STUDIES ON THE BIOTOXICITY OF THE MANGROVE VEGETATION ON THE FINGERLINGS OF *LIZA MACROLEPIS, TILAPIA MOSSAMBICA* AND *CHANOS CHANOS*

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work carried out by Shri. **K. MADHU** under my supervision and that no part thereof has been presented before any other degree.

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PREFACE

Mangroves are vegetation of trees and bushes growing in the brackish, saltish and muddy swamps along the coast and the estuarine banks. Of these, more than 70% of the mangroves are found in tropical and sub-tropical regions. In India, nearly 85% of mangroves are found in West Bengal and islands of Andaman and Nicobar as a specialised flora adopted for growing in saline muddy areas. The mangrove ecosystem has been colonised by both terrestrial and marine fauna with its complex canal, the plants, pneumatophores and aerial roots. The mangrove ecosystem offered an ample avenue of protection to the juvenile organisms. The ecosystem is rich in organic matter from the fallen and decayed leaves, as well as by the influx of nutrients from the sea and land drainage. Thus, it serves as ideal nursery ground for many aquatic species and few terrestrial species and these organisms depend on the rich organic detritus for their food.

The mangrove ecosystem has been gaining more importance in coastal fisheries as well as aquaculture. No other plant community in the world has, perhaps, attracted more scientific attention than the mangroves.

In the recent years, studies have been conducted for proper understanding of the ecosystem and its importance to fishery as well as aquaculture activities. These studies were mostly confined to the physical, chemical and biological characteristics of the waters and sediments of the ecosystem. Nutritional value provided by the mangrove vegetation to aquatic organisms has also been studied.

No work has so far been carried out on the toxicity of the mangrove vegetation on the aquatic organisms which inhabit these regions. A knowledge of the toxicity of the mangrove vegetation on fishes of the region is of very great importance in fisheries and mariculture. The present studies were undertaken for understanding the species of mangroves which adversely affect the fishes. The results of such studies would also be useful for providing cheaper materials from the mangrove vegetation to eradicate undesirable organisms from aquaculture ponds.

In these studies various parts of each mangrove species have been subjected to bio-assays on toxicity to Liza macrolepis., Tilapia mossambica and Chanos chanos fingerlings. The biochemical studies of the tissues of the dead fishes in comparison with the untreated ones were also conducted. Further, paper chromatographic studies also have been carried out to understand the number of individual chemical components present in each part of the plant material. In all seven mangrove species have studied.

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INTRODUCTION

Mangroves are defined as those trees and shrubs growing between high water of spring tide and the mean sea level where the wave action is feeble (Macnae,1968). The term mangroves is generally used for both the vegetative colonisation as well as individual species. Singh et al. (1986) defined mangroves as characteristic littoral plant formations of tropical and subtropical protected coast lines. They are salt tolerant forest ecosystems, of tropical and subtropical intertidal regions of the world. The distribution and restriction of mangroves in the sub-tropical regions are influenced by the pattern of warm water and cold water currents. Soft mud, sand bars and protected topography of the bays, lagoons and estuaries provide shelter from wave action and thereby enhance luxuriant growth of mangrove vegetation. The geographical occurrance of the mangroves are found in the West Africa, Atlantic U.S.A., Pacific America, East Africa, Australia, Asia and Oceanica, (Krishnamurthy et al., 1979 and Blatter, 1905).

In the Indian sub-continent as well as in the islandsof Andaman-Nicobar, the extent of mangrove forest along with their utility and silviculture management are outlined in the account of Chengappa (1944), Mathauda (1959), Rao (1950), (1959), Sidhu (1963), Thothathri (1960) and Venketeswaran(1968).

The ecological aspect of individual mangrove species and the ecology of particular regions along the Indian coast have been studied by Navalkar (1959) and Blasco(1975). In India, extensive areas of mangroves are found in West Bengal, Krishna, Godavari Delta, Cauveri Delta, Vembanad Lake region,

Kali Nadi Delta, Goa, around Bombay, Gulf of Cambay and Andaman and Nicobar islands.

The general features of mangrove species regarding the floral association, zonation , succession, soil and tidal features have been studied by Chapman (1970 & 1975), Werger (1978), Salomon (1978), Lugo and Snedaker (1974), Walsh (1974), Snedaker and Samuel (1978) and Lugo (1980). Savage (1972) has dealt with the taxonomy, identification, growth, development, zonation and artificial propagation of Floridian mangroves.

The biology of mangroves and their seedlings were studied by Roxburg (1814), Clark (1896) and Prain (1903). Estuarine mangroves on the West Coast of India has been described by Prain (loc. cit.), Tampi (1969), Mudaliar (1954), Navalkar (1956), Navalkar and Bharucha (1948) and Venketeswaralu (1944). Navalkar (1967) gives detailed picture of the mangrove ecosystems of the Bombay region, with the succession pattern. Untawale et al. (1973 & 1976) have extensively studied the mangroves of the Goa coast, while Joshi (1975) gives an account of the ecology in relation to the ionic intake in the case of mangroves of Tarekkol and Vasishti rivers. The tidal factors, still dynamics and vegetation of the mangroves of Barbulanga tidal estuary in Orissa were studied by Rao and Mukherjee (1972). An account of West Coast mangroves flora has been published by the Dwivedi et al. (1975) and the general account of the Indian mangroves flora is given by Blasco (loc. cit.).

The mangrove vegetation has been extensively described by different authors (loc. cit.). 27 species of mangroves have been recorded in India by Navalkar (1973). They have been classified into two groups "Swampy

mangroves" situated below the level of high tide and covered by sea water twice or once a day and "Tidal mangroves" submerged by spring tides and during cyclones, Quereshi (1954). Dwivedi et al. (loc. cit.) divides mangroves in to two groups, namely, the fringing mangrove of estuary and mangrove swamps around backwater area. Rajagopalan et al. (1980) describes the mangrove area of Cochin backwaters as formative and passive distribution.

The edaphic and environmental characteristics such as tidal factors have not been studied much. The prominent features of the mangrove soil is the presence of marine salts, along with its characteristic physical property, light insufficient or practically nil drainage, imersion under saline conditions etc., Macnae (loc. cit.). The high nutrient along with low wave action and absence of premonsoon currents near the banks leads to favourable conditions for the growth of mangroves (Untawale, loc. cit.). Mangroves usually flourish in fine alluvial mud composed mainly of silt and clay particles. These muds provide soft substratum essential for the anchorage of young seedlings with their root system developing and retaining moisture efficiently. Macintosh (1981), Untawale and Parulekar (loc. cit.) describe the estuarine mangroves of Goa as typical estuarine ecosystem with strong monsoon and tidal influence. Here heavy precipitation lowers the salt concentration considerably and increase the sediment load.

The physical factors, chemical factors and chloride content of the mangrove soil have been studied by Bharucha & Navalkar (1942), Navalkar (1942 & 1949). Naidoo & Raiman (1982) studied mangrove soil of South Africa

and concluded that soil is generally rich in clay, organic matter, catonic exchange capacity, Al, Fe and exchangeable bases.

Mangrove zonation has been attributed to frequency of tidal inundation, the salinity of interstitial water and water logging soil, Watson (1928), Macnae (loc. cit.). The occurrence of species or mixture of species involve tidal sorting of propagules, salinity preference, tolerance, interstitial salinity, competition for light in a developing stand, predation, parasitism and especially opportunism in relation to propagule availability, Teas (1979). Zonation has also been attributed to the changes in pH and salinity, Joshi (loc. cit.).

The physico-chemical factors of soil in relation to halophytic growth especially from the autecological point of view has been attempted only on the salt marsh species, namely Spartina alterniflora and S. anglica, (Chalmers, 1981; Piggot, 1969; Valeila and Teas, 1974; Nestler, 1977 and Howers et al., 1981).

In halophytes all cells actively absorbed K^+ and chloide ions, (Gutknecht & Dainly, 1969). The Na^+ ion concentration affect the halophytes more than that of K^+ ions (Joshi, loc. cit.). The capacity to regulate the uptake of the above ions can possibly affect the limit of mangrove distribution, (Joshi, loc. cit; Ranwell, 1974).

The microflora as well as heterotrophic bacterial flora of the mangroves swamps of Mandovi-Zuari estuary in Goa have been studied by Matonodkar et al. (1981). The microbiological study of the mangrove swamps in Killai backwaters have been conducted by Venkatesan and Ramamurthy (1971),

who reported the presence of physiological active group of bacteria. 21 bacterial species have been isolated from mud and water of the Sindhu dung and Malvan area in Maharshtra by Humnadkar and Agate (1985). Chandrika et al. (1985) describes the green sulphur bacteria responsible for detritus composition from mangrove mud of Karuthedam near Cochin. Mini Raman (1986) has studied the microbial flora in the Rhizosphere of Acanthus ilicifolius and Surendran (1985) the heterotrophic bacteria of mangrove ecosystem of the Cochin backwaters. Meenakshy (1985) has studied the germination and growth of Avicennia officinalis while the colonization of the Acanthus ilicifolius in the mangrove of the sea accreated region has been studied by Muralidharan (1984).

Biochemical as well as the chemical composition of the mangrove leaves of Goa and Maharashtra region have been studied by Bhosale et al. (1976), Kotmire and Bhosale (1979). Joshi et al. (1974)a & b have reported the mineral components of mangrove of Ratnagiri. Their studies showed that leaves are rich in protein, lipid and carbohydrate content and that their high calorific content makes them a source of nutrition to animal feeding on the decayed leaves. Untawale et. al. (1980) have studied the seasonal variation in heavy metal concentration in the mangrove foliage of Goa and found that the values are very high in comparison with that observed by Morton (1965) on Rhizophora Mangale in Florida. Gullati et al. (1979) have estimated the concentration of uranium, boron, nitrogen, phosphorous and potassium in the leaves of mangroves of the Goa region and found their content to be higher than in the terrestrial plants.

Achuthankutty and Sreekumar (1980) observed that maximum abundance of Penaeid population was sustained in the Goa mangroves during pre-monsoon season. The importance of mangrove in the marine fish production especially penaeid prawns have been reported earlier by Martosubroto and Naamin(1977), Snedakar (loc. cit.). Saly Anee Thomas (1985) has found that feeds compounded with mangrove leaves upto 30% and decomposed leaves upto 25% of the four species of mangrove vegetation namely, Rhizophora mucronata, Avicennia officinalis, Acanthus ilicifolius, Bruguiera Gymnorhiza in the diet of P. indicus had yielded good growth response.

The toxicity of the mangrove vegetation have not been scientifically studied and reported so far. The mangrove vegetation provide shelter for juvenile fishes. The fishes belonging to the family Mugilidae, Channidae, Clupidae, Pomadasyiae and Gerridae are usually found and harvested from the mangrove regions. Therefore, it was thought worthwhile to make a study of the bio-toxicity of the mangrove plants on fish fingerlings of Tilapia mossambica, Liza macrolepis and Chanos chanos.

The following mangrove species have been taken up for this bio-toxicity study. Acanthus ilicifolius (Linn), Acrostichum **aureum**, Avicennia officinalis (Blume), Brugueiera cylindrica, Clerodendrum inerme (Gaertn), Excoccaria agallocha (Linn) and Rhizophora mucronata (Lamk). A glance through the Ayurvedic literature (Chopra et al., 1956, Kirthikar & Basu, 1976. and Nadkarni, 1954) shows that these plants were used as drugs from olden days in the indigenous medical system.

Acanthus ilicifolius (Linn) (F. ACANTHACEAE)

Leaves are used in rheumatism and neuralgia, plant is used in asthma, decoction of the plant in dyspepsia and the leaves and tender roots in snake bite · Dymock et al. (1890) reported that the plant contains resins, alkaloid and organic acids. The plant shows astringent property and is a nervine tonic, expectorant and stimulant. Tender shoots and leaves ground small and soaked in water are applied to snake bites.

Avicennia officinalis (Blume) (F. VERBENACEAE)

It is used for the astringent and aphrodisiac properties of the bark. The unripe seeds are used as poultice to hasten suppuration of boils and abscesses. The bark has been reported to contain 2.5% tannin. It is used for small pox in Madras.

Clerodendrum inerme (Gaertn) (F. VERBENACEAE)

Juice of leaves and roots are used as alterative in scrofulous and venereal diseases. The root is also used in the form of liniment in rheumatism. The leaves contain amorphous bitter principle, resin and gum. Berries or root is administered to human poison caused by eating unwholesome fishes.

Excoccaria agallocha (Linn) (F. EUPHORBIACEA)

Milky juice of the plant boiled in oil is used in rheumatism, leprosy and paralysis. The decoction of leaves is used in epilepsy and applied to heal ulcers. The bark is used as emetic and purgative. Root used as an ingredient of embrocations used for the swelling of hand and feet. The latex of the plant shows

purgative and abortifacient property and is also a fish poison. The latex is acrid by nature. Tejbala, a soft reddish substance obtained from the lower part of trunk and roots of the plant is reputed as an aphrodisiac. The fresh milky juice of the tree is very acrid and injurious to the eyes and hence it is called "the blinding tree of India". It is poisonous to human beings. It is also used as adjunct to arrow poison (Chopra et al., 1965).

Rhizophora mucronata (Lamk) (F. RHIZOPHORACEAE)

The bark of the tree had been used for its astringent property, for haemorrhage, angina and cure for diabetis. The plant contains tannins.

Each part of the plant material such as flowers, seeds, leaves, stem bark and root bark were taken up for study separately. In each case the ethanolic solubles and the aquoeus solubles were studied for the toxicity on fingerlings of Tilapia mosambica, Liza macrolepis and Chanos chanos.

Biochemical studies on the variation of the total free sugar, total protein and cholesterol content in the muscle tissue of fish specimen showing lethality were estimated. These parameters were compared with those of the muscle tissue of the control fish.

Paper chromatographic studies of the ethanolic extract using various solvent systems have also been conducted to have an idea of the number of individual chemical components in each part of the plant materials.

MATERIALS AND METHODS

Fresh plant materials of different mangrove species were collected during the months of January and February 1989, from the Puduvypeen, Vypeen and Narakkal in Kerala State. Avicennia officinalis (dwarf variety) was collected from Karapad near Tuticorin, in Tamil Nadu. Seeds, flowers, leaves, stem bark, stem and root bark of each species were collected in sufficient quantities according to their availability. Identification of the mangrove species were made on their morphological characters with taxonomic descriptions, Gamble (1915), Figures 1a & 1b show the areas of collection and plates 1a-7b show the various species of mangroves collected.

The collected materials were brought to the laboratory and their fresh weight determined.. In the case of stem and root their barks were peeled off fresh and their weights also noted. All these materials were spread out and kept in the laboratory at room temperature free from sunlight for a period of 30 days. The dry weight of the parts were noted and the moisture content calculated.

The dried materials were coarsely powdered in a pulveriser and separately packed and kept for further treatment.

Ethanol Extraction

The dried powdered material was then extracted with hot ethanol using an all glass Soxhlet apparatus until the ethanolic extract is colourless. The ethanolic solution thus obtained was distilled using an all glass distillation apparatus on a water bath and finally concentrated and evaporated to dryness

Fig. 1a.

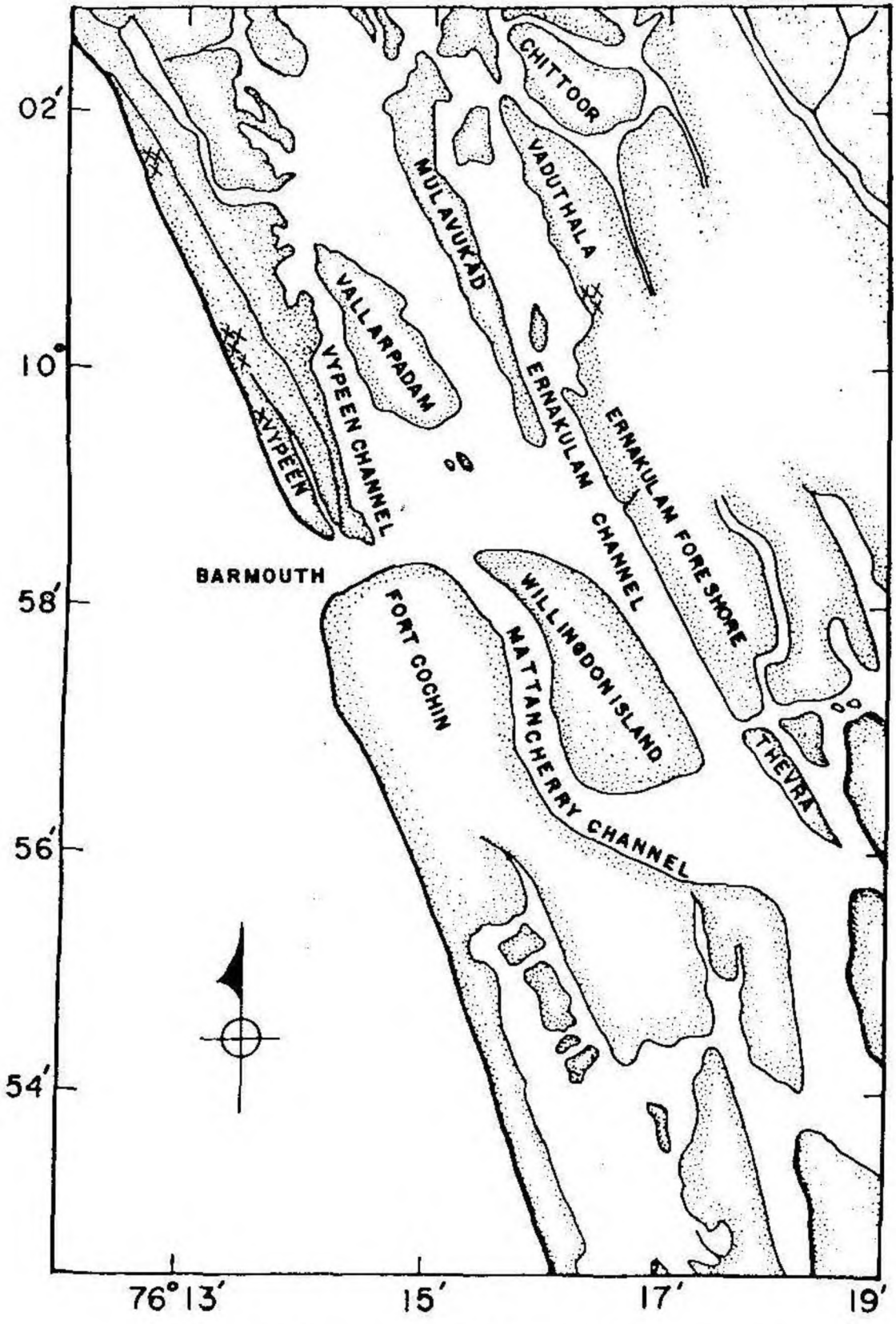

Fig. 1a - X - Site of collection of Mangrove spp.

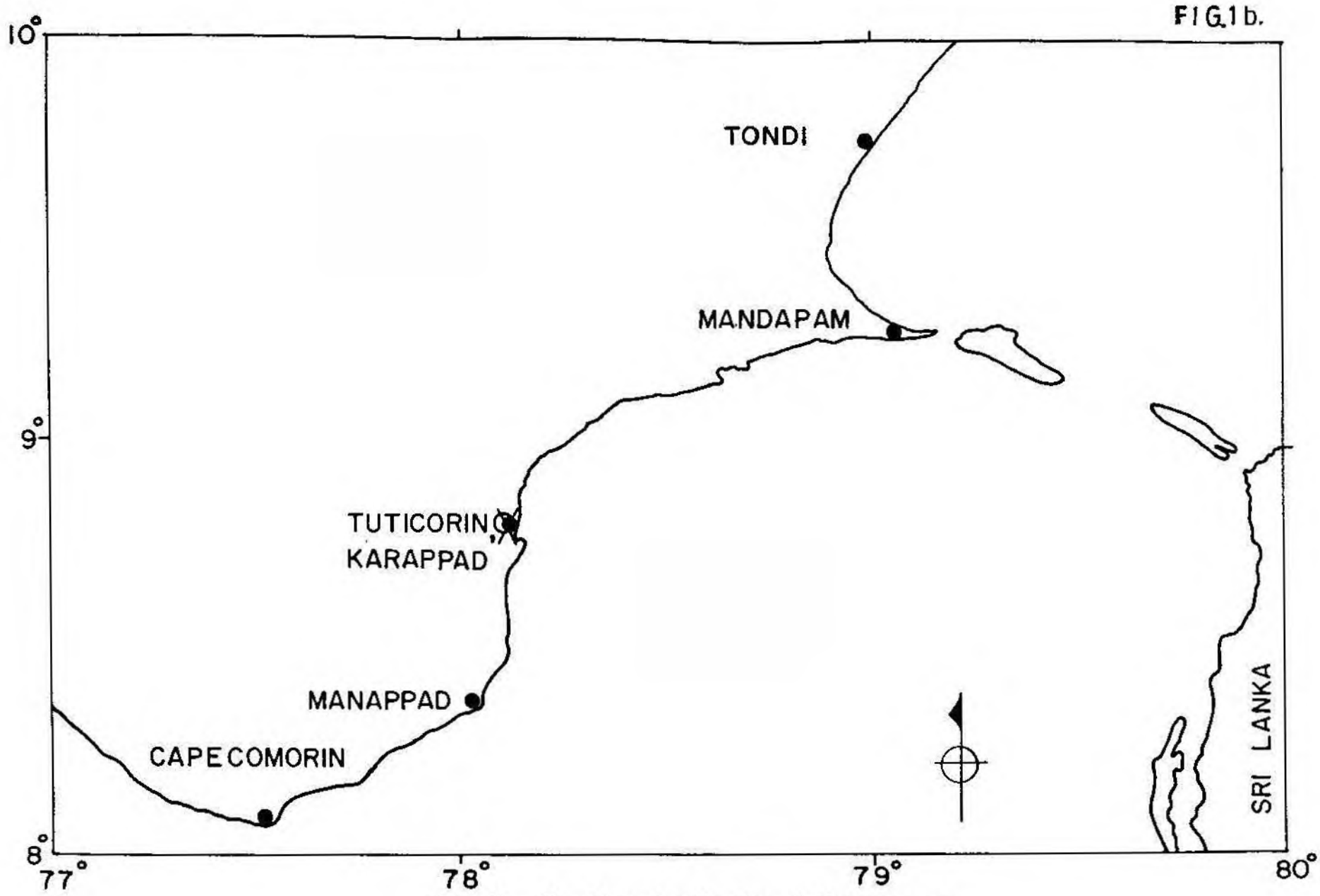

Fig. 1b - X - Site of collection of Mangrove spp.

PLATE. 1a. Acanthus *ilicifolius* (collected from Cochin).

PLATE. 1b. Structural diagram of A. *ilicifolius*.

PLATE - 1a

PLATE - 1b

PLATE 2. Acrosticchum aureum (collected from Cochin).

PLATE - 2

PLATE 3a. Avicennia officinalis (collected from Cochin).

PLATE 3b. A. officinalis (Tree Habit) (collected from Cochin).

PLATE - 3a

PLATE -3b

PLATE 3c. Avicennia officinalis (collected from Tuticorin).

PLATE 3d. Large single species colony of A. officinalis at Tuticorin.

PLATE - 3c

PLATE - 3d

PLATE 4. Bruguiera cylindrica (collected from Cochin).

PLATE - 4

PLATE 5a. Clerodendrum inerme (collected from Cochin).

PLATE 5b. Structural diagram of C. inerme.

PLATE - 5a

PLATE - 5b

PLATE 6a. Excoccaria agallocha (collected from Cochin).

PLATE 6b. Structural diagram of E. agallocha.

PLATE - 6a

PLATE - 6b

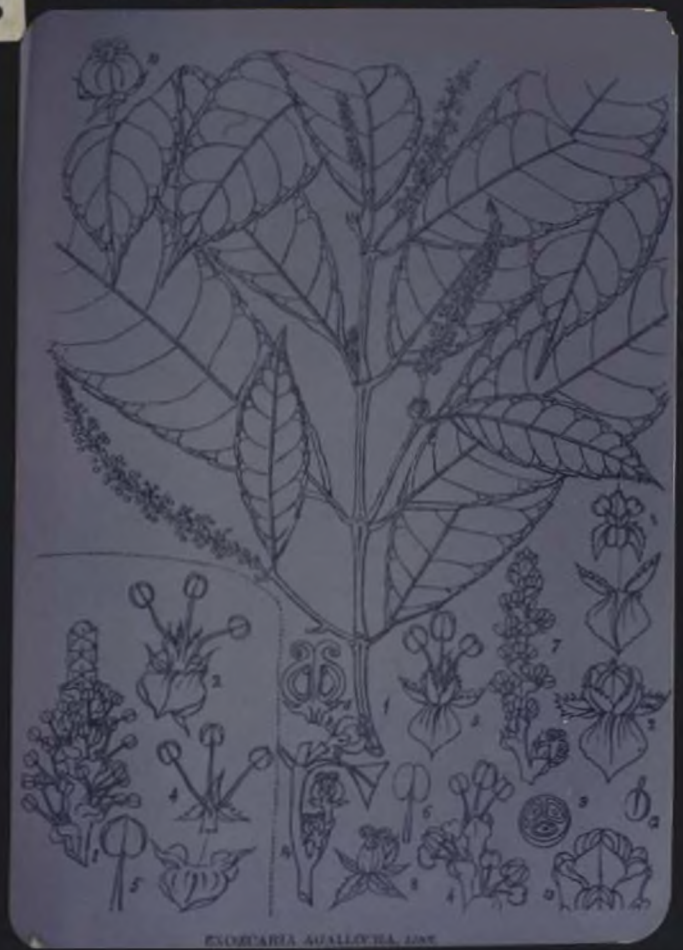

PLATE 7a. Rhizophora mucronata (collected from Cochin).

PLATE 7b. Structural diagram of R. mucronata.

PLATE - 7a

PLATE - 7b

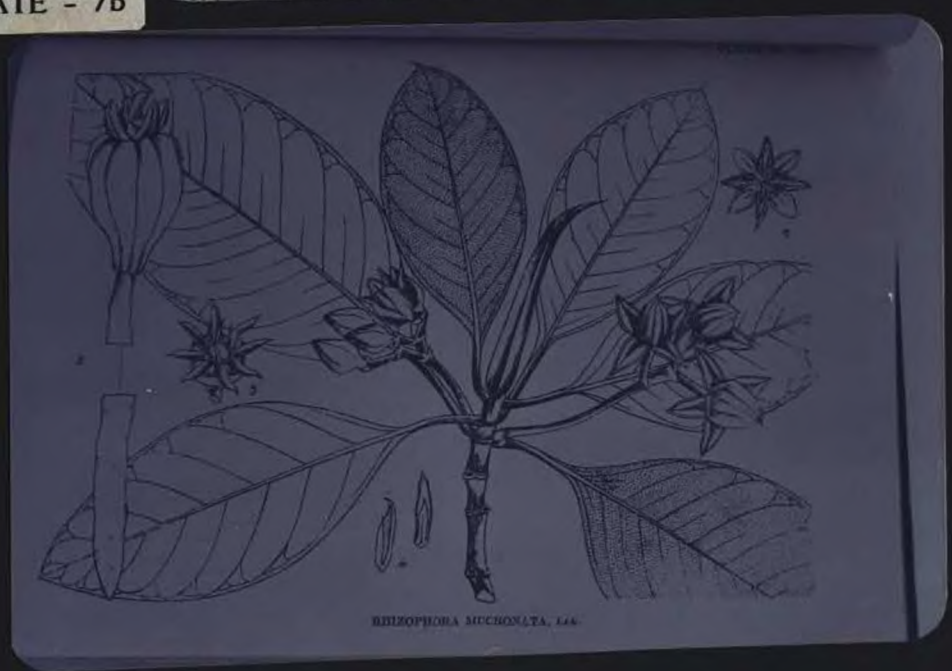

PLATE 8a. All glass Soxhlet apparatus - extraction of the dried powder of the parts of mangrove.

PLATE 8b. Distillation apparatus - distillation of ethanol extracts of mangrove spp.

PLATE - 8a

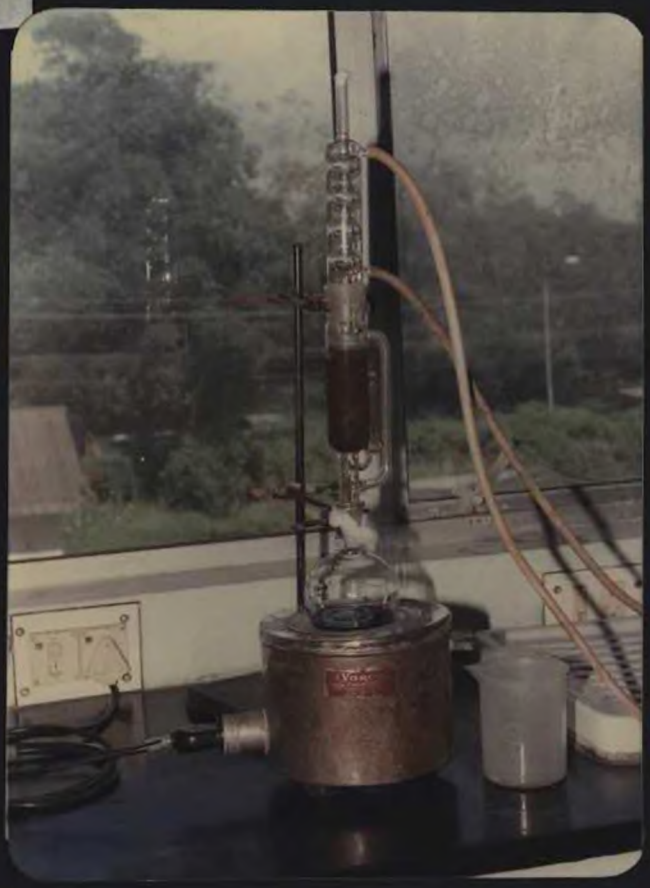

PLATE - 8b

PLATE 9a. Extracts of mangrove spp. after distilled.

PLATE 9b. Shandon apparatus.

PLATE - 9a

PLATE - 9b

at temperature of boiling water bath. The residue was dried in a dessicator over fused Calcium chloride for a period of 48-72 hours till a constant weight is obtained. The percentage weight of ethanol solubles of each plant material was calculated.

The dried residue of various plant materials were dissolved in 100 ml of ethanol with stirring using glass rod and warming over water bath where ever necessary and an aliquot volume of the cooled ethanolic solution equivalent to 2 gms of dried plant material pipetted out into clean dry boiling tubes. The ethanolic solution in the boiling tubes was evaporated to dryness using a water bath in each case and dissolved in 10 ml of phosphate-buffered-saline solution, which was prepared as given below.

Preparation of Phosphate-Buffered-Saline (P.B.S)

0.028M solution of dihydrogen phosphate was prepared in 1 litre of distilled water. The pH of this solution was adjusted to 7 by addition of 0.084M solution of potassium dihydrogen phosphate, little by little, and observing the pH after each addition. The volume of this mixture was determined using a measuring cylinder and to this an equal volume of 0.286M sodium chloride solution in distilled water was added and mixed thoroughly to give, Phosphate-buffered-saline.

Collection of fish fingerlings from Kerala agricultural University Fish Farm at Puduvypeen in Kerala State. 100 live specimens of mullet fingerlings (Liza macrolepis) of size, 45 mm, weight in average, 0.75 gm were collected

and brought to laboratory and kept alive in brackish water of salinity near about 5 ppt contained in polythene aquarium tank and acclimatized for thirteen days, with sufficient aeration. The water in the tank was renewed every alternate day, 100 live Tilapia mossambica fingerlings of average size,60 mm,weight,0.98 gm, and 100 live Chanos chanos fingerlings of average size,50 mm, weight,0.68 gm, were also collected, acclimatised and kept alive in the manner as in the case of Liza macrolepis fingerlings.

Toxicity Experiments On Fish Fingerlings

The three species of the acclimatized fish fingerlings viz. Liza macrolepis, Tilapia mossambica, Chanos chanos were used for the toxicity bio-assays. One test fish fingerling each was placed in finger bowl with 250 ml of saline water to which the test fish had been acclimatised and to which the ethanolic extract residue dissolved in 10 ml of PBS solution as given above was added and mixed thoroughly(Bakus, 1974). The test was repeated thrice with the extract of each part of plant material along with control experiment (blank) with same volume of acclimatised water. The time at which it dies was recorded. The experiments were terminated when fish showed constantly normal behaviour or death. Violent escape behaviour, paralysis and loss of equilibrium were also noted, if any. Experiments were conducted at the laboratory temperature (26.5°-32°C)

Test for total free sugar, total protein and cholesterol

In the experiments where the lethality was observed, the dead fish fingerlings were subjected to biochemical analysis of muscle tissues for estimation of total free sugar, total protein and cholesterol. These analyses

were also done with the control fish for comparison. In each case, the estimation was repeated six times and the mean of these was taken for comparison.

Total free sugar

Carbohydrate in the tissues of fishes exist as free sugar and as bound with protein. The total free sugar in the muscle of the dead specimen was estimated by the anthrone method.

The free sugars in muscle consist of mono-, di- and oligo- saccharides. The concentrated sulphuric acid in the antrone reagent hydrolyse di- and oligo-saccharides into mono-saccharides and dehydrates all mono-saccharides into furfural derivatives. These two compounds react with number phenolic compounds and one such is anthrone which produce a complex coloured product. The intensity of this is proportional to the amount of saccharides present in the sample, (Roe, 1955).

Total protein

Folin-Ciocalteu method (Lowry et al.,1951) was followed for the estimation of total protein in the tissues of the dead fish specimen.

The principle of this method is that the carbamyl groups of the protein molecules react with copper and potassium of the reagent to give a blue coloured copper potassium-biuret complex. This complex together with tyrosine and phenolic compounds present in the protein reduce the phosphomolybdate of the Folin reagent to intensify the colour of the solution.

Total cholesterol

The cholesterol of the tissues reacts in acetic acid with ferric chloride and sulphuric acid giving a red colour, Henly (1957). The intensity of colour is proportional to the amount of cholesterol present in the tissue. This method was followed for the estimation of cholesterol.

AQUEOUS EXTRACTS

2 gm. of dry powdered material of each part of mangrove species were heated with 10 ml. of distilled water in a boiling tube on a boiling water bath for about 1/2 an hour, cooled to room temperature and filtered through a plug of cotton wool to get the clear aqueous extract.

The toxicity bioassays on fingerlings of Liza macrolepis, Tilapia mossambica and Chanos chanos were repeated in the same manner as given for the ethanolic extract above and the results noted.

The estimation of total free sugar, total protein and cholesterol were also repeated with the muscle tissue of the dead fingerlings in the same manner as given for the ethanolic extract above. The results were tabulated.

PAPER CHROMATOGRAPHY

Ethanolic extract of each part of the mangrove species was chromatographed on Whatman filter paper (No.1) using Shandon apparatus. The

chromatogram was developed by the descending method. (Zweig & Whitaker, 1971). Five different solvent systems were used for developing the chromatograms. These solvents systems are given below.

1. Solvent : n-Butanol : Acetic acid : water = 14:4:50 v/v (Floch 1964, 1965)

2. Solvent : n-Butanol : Acetic acid : Water = 4: 1: 1 v/v (Zweig & Whitaker, loc. cit.).

3. Solvent : n-Butanol : Acetic acid : Water = 4:1:5 v/v (Bate-Smith, 1949., Gaffney et al., 1954; Smith, 1955).

4. Solvent : n-Butanol : Water : Ethanol = 5:4:1 v/v (Block et al., 1958)

5. Solvent : Benzene : Methanol : Water = 4:4:1 v/v (Ritter & Hartel, 1958)

These solvent systems are being used to identify the characteristic groups of organic compounds as given in the table below (Zweig & Whitaker, loc. cit).

	Solvent Systems	Used for separation of
1.	n-Butanol : Acetic acid : Water (14 : 4: 50 v/v)	Aliphatic and aromatic amines Cathecholamines and their derivatives.
2.	n-Butanol : Acetic acid : Water (4 : 1: 1 v/v)	Indoleamines and related compounds
3.	n-Butanol : Acetic acid : Water (4 : 1 : 5 v/v)	Aromatic acids, phenols and related compounds
4.	n-Butanol : Water : Ethanol (5 : 4: 1 v/v)	α -keto acids and derivatives
5.	Benzene : Methanol : Water (4 : 4 : 1 v/v)	Steroids

The various spots developed in the chromatograms were detected by illumination with Ultra-violet light. The dark and flourescent areas were encircled with a pencil and R_f values calculated. After this, chromatograms were exposed to ammonia vapour in the closed chamber of the Shandon apparatus for about 6 hours and then any new coloured areas observed were encircled with the pencil and their R_f values also calculated.

RESULTS

The data obtained in this study have been presented in the form of Tables.

Table 1 shows the percentage moisture content and ethanol solubles in each part of the mangrove plants taken up for study. In the case of Acanthus ilicifolius highest moisture content of 74.653% was found in flowers and lowest in the leaves (64.686%). In the case of Acrostichum aureum the moisture content was slightly higher (61.408%) in leaves compared to stem (60.000%). Avicennia officinalis stem showed lowest moisture content (42.537%) where as higher percentage was noted in leaves (60.80%) and flower (60.30%). The leaves of A. officinalis dwarf variety from Tuticorin had higher moisture content (76.73%). Leaves of Bruguiera cylindrica (69.65%) contains highest moisture percentage compared to seeds (58.44%) and stem bark(54.27%). The leaves of Clerodendrum inerme had highest moisture content(71.80%) whereas the flower contains moisture 65.22% and stem 27.38%. Stem bark of Excoccaria agallocha contains less moisture (58.86%) compared to leaves 65.84%. Leaves of Rhizophora mucronata contains higher moisture content, 74.36%, whereas the seeds contained 56.95%, stem bark 42.87% and root bark 5.00%.

Stem bark of A. ilicifolius contained highest amount of ethanol solubles, 11.47%, compared to leaves 5.14%, seeds 4.76% and flowers 2.19%.

TABLE - 1

MOISTURE CONTENT AND WEIGHT OF ETHANOL SOLUBLES OF EACH PART OF MANGROVES

Name and parts of Mangrove species	Fresh wt. (gm)	Dry wt. (gm)	Moisture content (gm)	Moisture content (%)	Weight of specimen powder taken for extraction (gm)	Weight of ethanolic extract residue (gm)	Ethanol Solubles (%)
1) Acanthus ilicifolius							
Seeds	116.483	31.532	84.951	72.929	31.532	1.5	4.757
Flowers	477.510	112.032	356.478	74.653	36.532	0.8	2.189
Leaves	610.175	215.475	394.700	64.686	71.975	3.7	5.140
Stem	187.895	52.311	135.584	72.159	52.311	6.0	11.469
2) Acrostichum aureum							
Leaves	797.438	307.745	489.692	61.408	91.745	3.5	3.814
Stem	227.262	90.839	1366.423	60.000	37.439	0.5	1.335
3) Avicennia officinalis							
Flowers	286.717	125.000	161.717	60.300	76.400	7.2	9.424
Leaves	305.497	121.282	184.215	60.803	73.282	7.5	10.234
Stem	125.412	72.065	53.347	42.537	64.565	1.0	1.548
4) Avicennia officinalis (Dwarf)							
Leaves	575.000	441.210	133.790	76.730	62.210	6.0	9.644
5) Bruguiera cylindrica							
Seeds	133.556	55.510	78.046	58.436	55.510	9.1	16.393
Leaves	433.650	131.650	302.060	69.655	74.590	9.5	12.736
Stem bark	165.060	75.478	89.582	54.272	67.478	16.6	2.371
6) Clerodendrum inerme							
Flowers	28.825	10.025	18.799	65.217	10.025	1.0	9.974
Leaves	372.463	105.023	267.440	71.803	89.023	11.1	12.468
Stem	63.795	46.330	17.465	27.376	46.330	1.2	2.371
7) Excoccaria agallocha							
Leaves	360.803	123.268	237.535	65.835	47.568	2.6	5.465
Stem bark	257.609	105.972	151.637	58.863	36.472	1.0	2.741
8) Rhizophora mucronata							
Seeds	366.110	157.619	208.491	56.947	53.119	8.5	16.001
Leaves	507.480	130.136	377.344	74.356	35.136	12.5	35.576
Stem bark	175.000	100.000	75.000	42.857	74.500	8.0	10.738
Root bark	100.000	95.000	5.000	5.000	40.000	4.9	12.250

Leaves of A. aureum contained more percentage of alcohol solubles (3.81%) compared to the stem (1.34%) A. officinalis leaves had highest percentage of ethanol solubles (10.23%) whereas the flowers had 9.42% and stem 1.55%. The dwarf variety of A. officinalis leaves gave 9.64% of ethanol solubles. In the case of B. cylindrica, the seeds show highest percentage of ethanol solubles (69.39%) while the leaves yielded 12.74% and stem bark of 2.37%. C. inerme showed highest ethanol soluble contents in leaves (12.47%) while flowers yielded 19.97% and stem (2.59%) of ethanol solubles. E. agallocha stem bark contained 2.74% and leaves 5.47% of ethanol solubles. The highest content of ethanol solubles was shown in leaves of R. mucronata (35.58%) and lowest in the root bark, 12.25%, with 16.00% in seeds and 10.74% in stem bark.

Table 2 shows the physical nature of the ethanol extract of each part of the mangrove species. The seeds and bark of B. cylindrica and R. mucronata yielded dark brown or reddish brown ethanolic solutions. All the parts of B. cylindrica and R. mucronata such as seeds, leaves, stem bark, and root bark yielded extracts which foamed at the end of distillation as the water content increases.

Table 3 gives the result of toxicity of the ethanolic extract of each part of mangrove species to the fingerlings of Liza macrolepis and table-4 shows the toxicity of water extracts. The colour of resulting solutions when the PBS solution of the extract is added to the acclimatised water is also given in the table. In the case of ethanol solubles, lethality was shown by stem bark of B. cylindrica; flower of

TABLE - 2

THE PHYSICAL NATURE OF THE ETHANOLIC EXTRACTS

Name of species	Parts of species	Colour of ethanolic extract	Foaming nature during final evaporation
Acanthus ilicifolius	Seeds	Greenish brown	-
	Flowers	Greenish brown	-
	Leaves	Dark brown	-
	Stem	Dark green	-
Acrostichum aureum	Leaves	Dark Green	-
	Stem	Light green	-
Avicennia officinalis	Flowers	Dark green	-
	Leaves	Greenish red	-
	Stem	Greenish brown	-
A. officinalis (Dwarf)	Leaves	Greenish black	-
Bruguiera cylindrica	Seeds	Dark brown	Foams at the end
	Leaves	Greenish black	Foams at the end
	Stem bark	Dark reddish brown	Foams at the end
Clerodendrum inerme	Flowers	Greenish brown	-
	Leaves	Brownish green	-
	Stem	Greenish brown	-
Excoccaria agallocha	Leaves	Greenish brown	-
	Stem bark	Greenish red	-
Rhizophora mucronata	Seeds	Reddish brown	Foams at the end
	Leaves	Dark green	Foams at the end
	Stem bark	Reddish brown	Foam at the end
	Root bark	Reddish brown	Foams at the end

TABLE - 3

TOXICITY OF ETHANOLIC EXTRACT OF MANGROVES SPECIES TO FINGERLINGS OF LIZA MACROLEPIS

Name and parts of Mangrove species	Colour when PBS Solution of Ethanolic residue is added to acclimatised water	Reaction of the fish			Mean time for lethality
		Expt. Ist	Expt. 2nd	Expt. 3rd	
1) Acanthus ilicifolius					
Seeds	Yellowish green	Slow swimming, gasping, escaping behaviour, then normal behaviour.	Same as in the first experiment.	Same as in the first experiment.	-
Flowers	Pale yellow	Violent escaping behaviour, resting at the bottom, then normal behaviour.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Greenish yellow	Slight escaping tendency for 30 minutes, then continued normal	Vigorous escaping behaviour, jumping, resting at the bottom then continued normal.	Same as in the first experiment.	-
Stem	Light green	Vigorous swimming, slow swimming, vigorous gasping later continued normal.	Slow swimming, vigorous gasping then same as in the first experiment.	Same as in the first experiment.	-
2) Acrostichum aureum					
Leaves	Greenish yellow	Violent escaping behaviour, slow gasping, resting at the bottom, vigorous swimming, later continued normal.	Same as in the first experiment.	Slow swimming, gasping. Then same as in the first. experiment	-
Stem	Light yellow	Vigorous swimming, gasping, fish was mainly staying at the bottom, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
3) Avicennia officinalis					
Flowers	Pale yellowish Green	Slow swimming, gasping, resting at the bottom, violent escaping behaviour, later continued normal.	Same as in the first experiment.	Vigorous swimming, escaping behaviour, slow gasping, later continued normal.	-
Leaves	Greenish yellow	Slow swimming, gasping at the bottom, normal breathing, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Pale yellow	Vigorous swimming, violent escaping behaviour, jumping and restless, later continued normal.	Same as in the first experiment.	Slow swimming, restless, later same as in the first. experiment.	

Contd...

4) Avicennia officinalis (Dwarf)					
Leaves	Greenish yellow	Violent escaping behaviour, gasping, restless, later continued normal.	Slow swimming, resting at mid-water level, later quiet normal.	Same as in the second experiment.	-
5) Bruguiera cylindrica					
Seeds	Light orange	Slow movement, vigorous gasping, later violent escaping behaviour. After one hour fish became normal and remains so.	Violent escaping behaviour till 1½ hrs later same as in the first experiment.	Same as in the first experiment.	-
Leaves	Light greenish yellow	Slow swimming, resting, swimming laterally then normal.	Same as in the first experiment.	Vigorous swimming, gasping, resting, slow swimming then continued normal.	-
Stem bark	Orange	Vigorous swimming, gasping, violent escaping behaviour, jumping, swimming with lateral side, then swimming upside down after 30 minutes, later resting at the bottom, gulping, slight jumping, death at 55 minutes.	Upto 40 minutes same as in the first experiment then jumping, gulping and dying at 58 minutes.	Behaviour same in the first experiment and death at 53 minutes.	55 minutes 20 seconds
6) Clerodendrum inerme					
Flowers	Light yellow	Violent escaping behaviour, vigorous gasping, jumping and death at 2 hours 1 minute.	Same as in the first experiment. Death at 2 hrs. 10 minutes.	Same as in the first experiment. Death at 1 hour 58 minutes.	2 hours 3 minutes
Leaves	Greenish yellow	Violent escaping behaviour, gasping, mainly fish was staying at the bottom, later continued normal	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Greenish yellow	Restless, violent escaping behaviour, gasping, slow swimming, and resting. After 1 hour became normal.	Same as in the first experiment.	Same as in the first experiment	-
7) Excoccaria agallocha					
Leaves	Light yellow	Violent escaping behaviour, restless, vigorous, gasping, swimming, loss of equilibrium after 1 hour 24 minutes, jumping, lying on lateral side after 1 hour 30 minutes, slow gasping and gulping. Death at 2 hours 25 minutes.	Same as in the first experiment upto 1 hour 20 minutes, loss of equilibrium at 1 hour 38 minutes, then jumping, vigorous gasping, lying on lateral side after 1 hour 40 minutes. Death at 2 hours.	Same as in the second experiment. Death at 2 hours 15 minutes.	2 hours 13 minute 20 second

Contd.....

Stem bark	Green	Violent escaping behaviour, gasping, swimming was normal upto 20 minutes, then loss equilibrium and death at 42 minutes.	Same as in the first experiment with death at 45 minutes.	Same as in the first experiment. Death at 50 minutes.	45 minutes 40 seconds
8) Rhizophora mucronata					
Seeds	Reddish brown	Vigorous swimming, violent escaping behaviour, restless, resting at the bottom,lying on lateral side after 30 minutes,death at 48 minutes.	Same as in the first experiment. Death at 40 minutes.	Same in the first experiment upto 43 minutes,then swimming upside down. Death at 55 minutes.	47 minutes 40 seconds
Leaves	Brown	Slow swimming, gasping, resting upto 18 minutes, lying on lateral side after 48 minutes, vigorous swimming jumping,swimming up side down. Death at 2 hours 40 minutes.	Same as in the first experiment. Death at 2 hours 55 minutes.	Upto 1 hour 30 minutes vigorous swimming and restless. Later same as in the first experiment. Death at 2 hours 30 minutes	2 hours 41 minutes 40 seconds
Stem bark	Orange	Vigorous swimming,gasping,and gulping. After 30 minutes swimming upside down. Then quiet normal, resting at 40th minute . Loss of equilibrium, death at 1 hour 5 minutes.	Same as in the first experiment. Death at 55 minutes.	Same as in the first experiment. Death at 1 hour 15 minutes .	1 hour 5 minutes
Root bark	Light orange	Same as in the stem bark experiment. Death at 1 hour 45 minutes.	Same as in the first experiment. Death at 1 hour 30 minutes.	Same as in the first experiment. Death at 1 hour 15 minutes.	1 hour 30 minutes

- = No lethality was observed.

TABLE - 4

TOXICITY OF WATER EXTRACT OF MANGROVES SPECIES TO FINGERLINGS OF LIZA MACROLEPIS

Name & Part of Mangroves species	Colour when PBS solution of water extract is added to acclimatised water	Reaction of the Fish			Mean time for lethality
		Expt. 1st	Expt. 2nd	Expt. 3rd	
1) Acanthus ilicifolius					
Seeds	Pale yellow	Slow swimming, gasping, and escaping behaviour, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Flowers	Dark yellow	Vigorous swimming up to 15 minutes. Later fish became quiet normal.	Same as in the first experiment.	Slow swimming, vigorous gasping, resting, later quiet normal.	-
Leaves	Light yellow	Restless, vigorous swimming, resting. After 2 hours became normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Light yellow	Same as in the extract of A. ilicifolius leaves experiment.	Same as in the first experiment.	Same as in the first experiment.	-
2) Acrostichum aureum					
Leaves	Light green	Vigorous swimming, resting, gasping, then slow swimming later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Greenish yellow	Violent escaping behaviour, gasping, restless resting, later continued normal.	Same as in the first experiment.	Slow swimming, vigorous gasping, later quiet normal.	-
3) Avicennia officinalis					
Flowers	Dark Green	Slow swimming, gasping, resting up to 1 hour 30 minutes, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Green	Violent escaping behaviour, slow swimming later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Pale yellow	Vigorous escaping behaviour, gasping, backward and forward movements, slow swimming, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-

Contd.

4) Avicennia officinalis (Dwarf)					
Leaves	Green	Same as in the A. officinalis leaves extract experiment. (above).	Same as in the first experiment.	Same as in the first experiment.	-
5) Bruguiera cylindrica					
Seeds	Orange	Vigorous swimming, gasping, violent escaping behaviour, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Dark Orange	Slow gasping, gulping, forward and backward movements, later continued normal.	Vigorous escaping behaviour, gasping. After 2 hours same as in the first experiment.	Same as in the first experiment.	-
Stem bark	Orange	Violent escaping behaviour, gasping, swimming upside down then jumping, lying on lateral side. Death at 1 hour 10 minutes.	Same as in the first experiment. Death at 1 hour 15 minutes.	Same as in the first experiment. Death at 1 hour	1 hour 8 minutes 20 seconds
6) Clerodendrum inerme					
Flower	Yellowish green	Vigorous escaping behaviour, violent gasping, later resting for few minutes, loss of equilibrium after 1 hour 45 minutes, lying on the lateral side at 1 hour 55 minutes. Death at 2 hours 15 minutes.	Same as in the first experiment. Death at 2 hours 10 minutes.	Slow swimming, resting, gulping, later same as in the second experiment. Death at 2 hours.	2 hours 8 minutes 20 seconds
Leaves	Dark green	Vigorous swimming, slow gasping, resting and later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Yellowish green	Same as in the C. inerme leaves extract experiment.	Same as in the first experiment.	Same as in the first experiment.	-
7) Excoccaria agallocha					
Leaves	Orange	Slight escaping behaviour, gulping, gasping, jumping, loss of equilibrium, then violent swimming, lying on lateral side. Death at 2 hours 50 minutes.	Same as in the first experiment. Death at 2 hours 55 minutes.	Vigorous escaping behaviour, gasping, jumping, loss of equilibrium, later same as in the first experiment. Death at 2 hours 30 minutes.	2 hours 45 minutes
Stem bark	Pale yellow	Vigorous swimming, violent escaping behaviour, loss of equilibrium, upside down swimming, lying on lateral side. Death at 40 minutes.	Same as in the first experiment. Death at 50 minutes.	Violent escaping behaviour resting, lying on lateral side with gasping and jumping. Death at 55 minutes.	48 minutes 20 second

8) *Rhizophora mucronata*

Seeds	Orange	Vigorous swimming, violent escaping behaviour, resting slow swimming and resting, *loss of equilibrium, up side* down movements. Death at 1 hour 5 minutes.	Vigorous swimming for few minutes, resting, loss of equilibrium, lying on lateral *side, slight jumping and* gulping. Death at 1 hour 20 minutes.	Same as in the first experiment. Death at 1 hour 30 minutes.	1 hour 18 minutes 20 seconds
Leaves	Grayish	Violent swimming, vigorous *escaping behaviour, gulping* loss of equilibrium and death at 1 hour 30 minutes.	Same as in the first *experiment.* Death at 1 hour 40 minutes.	Same as in the first *experiment.* Death at 1 hour 35 minutes.	1 hour 35 minutes
Stem bark	Dark orange	Vigorous escaping behaviour *slow swimming, sluggish* movements, violent gasping, loss of equilibrium and death at 1 hour 30 minutes.	Same as in the first *experiment.* Death at 1 hour 15 minutes	Violent escaping beha-*viour, gulping and* lying on lateral side, jumping forward and backward movements. Death at 1 hour 40 minutes.	1 hour 28 minutes 20 seconds
Root bark	Orange	Same as in the R. mucronata stem bark extract experiment (above). *Death at 1 hour 15 minutes.*	Slow swimming, restless, gulping, resting, later loss of equilibrium and death at 1 hour 10 minutes.	Same as in the first experiment. Death at 1 hour 30 minutes.	1 hour 18 minutes 20 seconds

- = No lethality was observed.

C. inerme; leaves and stem bark of E. agallocha; seeds, leaves, stem and root bark of R. mucronata. In the case of water extract also the same results were obtained.

Table 5 & 6 show the results of toxicity bioassays of ethanol and water extracts of mangrove species to fingerlings of T. mossambica as well as the colours of the resulting solution in which fish is tested. The ethanol solution of leaves of A. aureum; stem bark of B. cylindrica; leaves and stem bark of E. agallocha and seeds, leaves, stem and root bark of R. mucronata showed lethality to T. mossambica fingerlings. The water extract of these parts of the mangrove also gave the same result.

Table 7 & 8 represents the result of toxicity test conducted with ethanol and water extract of mangrove species to the fingerlings of C. chanos. The colour of the resulting solution in which fish is tested is also given in these tables. The seeds and stem of A. ilicifolius; seeds, leaves and stem bark of B. cylindrica; flower and leaves of C. inerme; leaves and stem bark of E. agallocha and seed, stem and root bark of R. mucronata showed lethality to the fingerlings of C. chanos. Same part of mangrove species showed lethality of their water extract.

The ethanol and water extract of mangrove species other than those noted above for lethality produced behavioural disturbances to the fingerlings of L. macrolepis, T. mossambica and C. chanos. These disturbances are also given in table 3 to 8.

Tables 9a and 9b indicate the lethality and non-lethality of fishes to mangrove extracts. The lethality to all fishes were shown by

TABLE - 5

TOXICITY OF ETHANOLIC EXTRACT OF MANGROVES SPECIES TO FINGERLINGS OF TILAPIA MOSSAMBICA

Name & Part of Mangroves Species	Colour when PBS solution of Ethanolic residue is added to acclimatised water	Reaction of the fish			Mean time for lethality
		Expt. 1st	Expt. 2nd	Expt. 3rd	
1) Acanthus ilicifolius					
Seeds	Yellowish green	Vigorous escaping behaviour, slow gasping, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Flowers	Pale yellow	Vigorous escaping behaviour, restless, resting, jumping, gasping, later continued normal.	Same as in the first experiment.	Same as in the first experiment	-
Leaves	Greenish yellow	Same as in the A. ilicifolius leaves extract experiment (above).	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Light green	Vigorous escaping behaviour, violent gasping, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
2) Acrostichum aureum					
Leaves	Greenish yellow	Slow swimming, resting, vigorous, gasping, loss of equilibrium, lying on lateral side and death at 3 hours 50 minutes.	Vigorous escaping behaviour, gasping, loss of equilibrium, jumping, swimming with head inclined upward and death at 3 hours 25 minutes.	Same as in the first experiment. Death at 3 hours 55 minutes.	3 hours 43 minutes 20 seconds
Stem	Light yellow	Violent escaping behaviour, vigorous, gasping, gulping at equal intervals, later continuing normal.	Same as in the first experiment.	Slow swimming, resting in the mid water, later continued normal.	-
3) Avicennia officinalis					-
Flowers	Pale yellowish green	Vigorous swimming, violent escaping behaviour, restless and later continued normal.	Same as in the first experiment.	Same as in the first experiment.	
Leaves	Greenish yellow	Same as in the experiment with ethanolic extract of A. officinalis flowers.	Same as in the first experiment.	Same as in the first experiment.	-

Contd.....

Stem bark	Pale yellow	Slow swimming, resting, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
4) A. officinalis (dwarf)					
Leaves	Greenish yellow	Same as in the experiment with ethanolic extract of A. officinalis leaves.	Same as in the first experiment.	Same as in the first experiment.	-
5) Bruguiera cylindrica					
Seeds	Light orange	Slow swimming, jumping, lying at the bottom, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	
Leaves	Light greenish yellow	Violent escaping behaviour, gasping, resting at the bottom, later continued normal.	Same as in the first experiment.	Vigorous gulping, gasping, violent escaping tendency, later continued normal.	-
Stem bark	Orange	Slow swimming, sluggish resting, loss of equilibrium, jumping, swimming with head inclined upwards and lying on lateral side. Death at 3 hours 5 minutes.	Vigorous swimming, escaping tendency, later same as in the first experiment. Death at 3 hours 20 minutes.	Same as in the first experiment. Death at 3 hours 15 minutes.	3 hours 13 minutes 20 seconds
6) Clerodendrum inerme					
Flowers	Light yellow	Slow swimming upto 1 hour 30 minutes, resting and later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Greenish yellow	Violent escaping behaviour, gasping, gulping, resting at the bottom, later continued normal.	Slow swimming and gasping, later continued normal.	Same as in the first experiment.	-
Stem	Greenish yellow	Same as in the above experiment.	Slow swimming and gasping, later continued normal.	Same as in the first experiment.	-
7) Excoccaria agallocha					
Leaves	Light yellow	Violent escaping behaviour, gasping, gulping, loss of equilibrium, lying on lateral side, swimming upside down and death at 2 hours 50 minutes.	Distress and vigorous escaping behaviour, loss of equilibirum, lying on lateral side, and death at 2 hours 40 minutes.	Same as in the first experiment. Death at 2 hours 55 minutes.	2 hours 48 minutes 20 seconds
Stem bark	Green	Violent escaping behaviour, distress, loss of equilibrium, restless behaviour and death at 2 hours 25 minutes.	Same as in the first exeperiment. Death at 2 hours 30 minutes.	Swimming vigorously then slowly and later gradually increasing the swimming velocity and death at 2 hours 40 minutes.	2 hours 31 minutes 40 seconds

Contd....

8) Rhizophora mucronata

Seeds	Reddish brown	Vigorous swimming, violent escaping behaviour, distress, loss of equilibrium, lying on lateral side and death at 3 hours 5 minutes.	Moderate escaping behavious, loss of equilibrium, gasping, lying on the lateral side,swimming upside down, death at 2 hours 55 minutes.	Violent escaping behaviour,distress, loss of equilibrium, death at 2 hours 55 minutes.	2 hours 58 minutes 20 seconds
Leaves	Brown	Violent escaping behaviour, restless, gasping and loss of equilibrium. Death at 1 hour 30 minutes.	Slow swimming, resting, loss of equilibrium, swimming upwide down. Death at 1 hour 18 minutes.	Vigorous escaping behaviour, loss of equilibrium, gasping, and death at 1 hour 40 minutes.	1 hour 29 minutes 20 seconds
Stem bark	Orange	Violent escaping behaviour, loss of equilibrium lying on lateral side and death at 1 hour.	Same as in the first experiment. Death at 1 hour 15 minutes.	Same as in the first experiment and death at 1 hour 10 minutes.	1 hour 8 minutes 20 seconds
Root bark	Light orange	Violent escaping behaviour, distress, loss of equilibrium, swimming upside down. Death at 1 hour.	Same as in the first experiment. Death at 1 hour 15 minutes.	Moderate escaping behaviour, loss of equilibrium, and death at 1 hour 5 minutes.	1 hour 6 minutes 40 seconds

- = No lethality was observed

TABLE - 6

TOXICITY OF WATER EXTRACT OF MANGROVES SPECIES TO FINGERLINGS OF TILAPIA MOSSAMBICA

Name & Part of Mangroves species	Colour when PBS solution of water extract is added to acclimatised water	Reaction of the Fish			Mean time for lethality
		Expt. 1st	Expt. 2nd	Expt. 3rd	
1) Acanthus ilicifolius					
Seeds	Pale yellow	Almost normal behaviour, distress, rising to the surface restless during the first 30 minutes, later continued normal.	Moderate escaping behaviour, distress and restless, later continued normal.	Same as in the first experiment.	-
Flowers	Dark yellow	Almost normal behaviour except at times restless during the first 30 minutes and then normal.	Same as in the first experiment.	Almost normal behaviour except at times restless during the first 45 minutes and then normal.	-
Leaves	Light yellow	Same as in the experiment with the water extract of A. ilicifolius flowers.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Light yellow	Same as in the experiment with water extract of A. ilicifolius flower.	Same as in the first experiment.	Same as in the first experiment.	-
2) Acrostichum aureum					
Leaves	Light green	Violent escaping behaviour, distress, loss of equilibrium, swimming upside down, gasping vigorously and death at 3 hours 30 minutes.	Same as in the first experiment. Death at 3 hours 20 minutes.	Vigorous gasping, jumping, resting at the bottom swimming and lying on the bottom. Death at 3 hours 15 minutes.	3 hours 21 minutes 40 seconds
Stem	Greenish yellow	Almost normal behaviour except at times restless during the first 1 hour 20 minutes, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-

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3) Avicennia officinalis					
Flowers	Dark green	Almost normal behaviour except at times with moderately escaping behaviour during the first 55 minutes, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Green	Almost normal behaviour except at times restless during the first 1 hour 20 minutes, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Pale yellow	Same as in the experiment with water extract of A. officinalis leaves.	Same as in the first experiment.	Same as in the first experiment.	-
4) A. officinalis (Dwarf)					
Leaves	Green	Same as in the experiment with water extract of A. officinalis leaves.	Same as in the first experiment.	Same as in the first experiment.	-
5) Bruguiera cylindrica					
Seeds	Orange	Vigorous swimming, gulping, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Dark orange	Same as in the experiment with water extract of B. cylindrica seeds(above).	Same as in the first experiment.	Same as in the first experiment.	-
Stem bark	Orange	Violent escaping behaviour, restless, distress, loss of equilibrium, jumping, upside down movements, lying on lateral side and death at 3 hours 15 minutes.	Violent behaviour, distress, rising to surface loss of equilibrium, struggling and death at 3 hours 20 minutes.	Vigorous escaping behaviour, jumping, later same as in the first experiment. Death at 3 hours 15 minutes.	3 hours 16 minutes 40 seconds
6) Clerodendrum inerme					
Flowers	Yellowish green	Moderate escaping behaviour, almost normal behaviour except at times restless during the first 50 minutes and then continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Dark green	Same as in the experiment with water extract of C. inerme flowers.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Yellowish green	Same as in the experiment with water extract of C. inerme flowers.	Same as in the first experiment.	Same as in the first experiment.	-

Contd.....

7) Excoccaria agallocha					
Leaves	Orange	Violent escaping behaviour gasping, loss of equilibrium lying on lateral side,resting, gulping,struggling and death at 1 hour 2 minutes.	Slow escaping behaviour restless, lying on lateral side, struggling and death at 1 hour 20 minutes.	Same as in the first experiment. Death at 1 hour 30 minutes.	1 hour 17 minutes 20 seconds
Stem bark	Pale yellow	Same as in the experiment with water extract of E. agallocha leaves. Death at 2 hours 35 minutes.	Same as in the first experiment. Death at 2 hours 30 minutes.	Violent escaping behaviour, jumping, gulping and death at 2 hours 40 minutes.	2 hours 35 minutes
8) Rhizophora mucronata					
Seeds	Orange	Violent escaping behaviour upto 40 minutes, slow swimming, resting at the bottom, lying on the lateral side and death at 2 hours 5 minutes.	Same as in the first experiment. Death at 2 hours 15 minutes.	Same as in the first experiment. Death at 2 hours 30 minutes.	2 hours 16 minutes 40 seconds
Leaves	Grayish	Violent swimming upto 50 minutes, resting at the bottom, lying on the lateral side, movements with up side down and death at 3 hours 30 minutes.	Slow swimming, loss of equilibrium and then same as in the first experiment. Death at 2 hours 50 minutes.	Same as in the first experiment. Death at 3 hours 15 minutes.	3 hours 11 minutes 40 seconds
Stem bark	Dark orange	Slow swimming, resting on the bottom, restless and vigorous escaping behaviour, after 10 minutes loss of equilibrium and death at 40 minutes.	Violent swimming, escaping behaviour, same as in the first experiment. Death at 50 minutes.	Same as in the first experiment. Death at 1 hour.	50 minutes
Root bark	Orange	Violent escaping behaviour, gasping, resting, swimming, loss of equilibrium, swimming upside down and death at 1 hour.	Same as in the first experiment. Death at 1 hour.	Same as in the first experiment. Death at 1 hour 5 minutes.	1 hour 1 minute 40 seconds

- = No lethality was observed.

TABLE - 7

TOXICITY OF ETHANOLIC EXTRACT OF MANGROVES SPECIES TO FINGERLINGS OF CHANOS CHANOS

Name & Part of Mangroves species	Colour when PBS solution of Ethanolic residue is added to acclimatised water	Reaction of the Fish			Mean time for lethality
		Expt. Ist	Expt. IInd	Expt. IIIrd	
1) Acanthus ilicifolius					
Seeds	Yellowish Green	Violent escaping behaviour, distress, rising occasionally to surface, loss of equilibirium, jumping swimming upside down and death at 4 hours.	Same as in the first experiment and death at 4 hours 10 minutes.	Same as in the first experiment and death at 4 hours 5 minutes.	4 hours 5 minutes
Flowers	Pale Yellow	Almost normal behaviour except at times restless during the first 1 hour 20 minutes and then normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Greenish Yellow	Same as in the experiment with ethanolic extract of flower of A. ilicifolius.	Same as in the first experiment.	Same as in the firsy experiment.	-
Stem	Light Green	Vigrous swimming, violent escaping behaviour, slow swimming and restless upto 1 hour 55 minutes and then resting, slow swimming, lying on later at side, upside down swimming and lying gulping, jumping. Death at 4 hour 15 minutes.	Same as in the first experiment. Death at 4 hour 30 minutes.	Violent swimming jumping, lying on lateral side, loss of equilibrium, gulping and death at 4 hours 10 minutes.	4 hours 18 minutes 20 seconds.

.....2.

2) Acrostichum aureum					
Leaves	Greenish Yellow	Almost normal behaviour except at times restless during the first 30 minutes and then normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Light Yellow	Normal behaviour except the first one hour and then continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
3) Avicennia officinalis					
Flowers	Pale Yellowish Green	Almost normal behaviour during the first 2 hours 40 minutes and then continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Greenish Yellow	Vigorous escaping behaviour upto 30 minutes. Then resting, slow swimming, later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Pale Yellow	Normal behaviour.	Same as in the first experiment.	Same as in the first experiment.	-
4) Avicennia officinalis (dwarf)					
Leaves	Greenish Yellow	Same as in the experiment with the ethanolic extract of A. officinalis stem.	Same as in the first experiment.	Same as in the first experiment.	-
5) Bruguiera cylindrica					
Seeds	Light orange	Violent escaping behaviour loss of equilibrium, lying on lateral side, swimming upside down, lying at the bottom, gasping and death at 4 hours 25 minutes.	Moderate escaping behaviour, loss of equilibrium, lying on lateral side, jumping and resting alternately. Death at 4 hours 30 minutes.	Same as in first experiment and death at 4 hours 35 minutes.	4 hours 30 minutes

.....3.

Leaves	Light greenish Yellow	Violent escaping behaviour with jumping, swimming vigorously and gasping, lying on lateral side, *mainly staying at the* bottom. Death at 4 hours 20 minutes.	Slow swimming, resting, lying on the lateral side, gulping and death at 4 hours 15 minutes.	Vigorous escaping behaviour, gasping, jumping, resting and gulping. Death at *4 hours.*	4 hours 11 minutes 40 seconds
Stem bark	Orange	Moderate escaping *behaviour, gasping,* jumping and swimming upside down. Death at 4 hours.	Same as in the first *experiment.* Death at 4 hours 20 minutes.	Vigorous swimming, gasping, lying on the lateral side, gasping and jumping. Death at 4 hours 10 minutes.	4 hours 10 minutes
6) *Clerodendrum inerme*					
Flowers	Light yellow	Violent escaping behaviour jumping, swimming upside down and lying on the *lateral side.* Death at 4 hours 20 minutes.	Same as in the first experiment and death at 4 hours 13 minutes.	Same as in the first experiment and death at 4 hours 28 minutes.	4 hours 20 minutes 20 seconds
Leaves	Greenish yellow	Vigorous escaping behaviour, jumping, loss of equilibrium, alternately jumping and swimming and gasping. Death at 4 hours 20 minutes.	Slow escaping behaviour, distress, resting and swimming on lateral side. Death at 4 hours 10 minutes.	Jumping violent escaping behaviour and death at 4 hours.	4 hours 10 minutes
Stem	*Greenish yellow*	Almost normal behaviour except at times restless during the first 2 hours 30 minutes, later continued normal.	Almost normal behaviour except at times restless during the first 1 hour 5 minutes, later continued normal.	Same as in the first experiment.	-
7) Excoccaria agallocha					
Leaves	Light yellow	Violent escaping behaviour, distress loss of equilibrium, swimming upside down, lying at the bottom and slight jumping. Death at 1 hour 5 minutes.	Same as in the first experiment. Death at 1 hour 2 minutes.	Slight escaping behaviour upto 5 minutes, loss of equilibrium, lying on *lateral side and slow* gasping. Death at 1 hour.	1 hour 2 minutes 20 seconds
Stem	Green	Violent swimming, escaping behaviour, loss of equilibrium and death at 2 hours 48 minutes.	Same as in the first experiment. Death at *3 hours.*	Slow swimming, gasping, jumping. Death at *3 hours 5 minutes.*	2 hours 57 minutes 40 seconds

.....4.

8) Rhizophora mucronata

Seeds	Reddish brown	Vigorous swimming, violent escaping behaviour, loss of equilibrium and death at 2 hours 55 minutes.	Same as in the first experiment. Death at 2 hours 48 minutes.	Slow swimming, gasping, resting, loss of equilibrium and death at 3 hours.	2 hours 54 minutes 20 seconds
Leaves	Brown	Violent escaping behaviour upto 25 minutes, later continued normal.	Same as in the firtst experiment.	Slow swimming, gasping upto 30 minutes, later continued normal.	-
Stem bark	Orange	Violent escaping behaviour, loss of equilibrium, lying on lateral side, jumping, rising to surface and death at 3 hours 5 minutes.	Same as in the first experiment. Death at 3 hours.	Same as in the first experiment. Death at 3 hours 15 minutes.	3 hours 6 minutes 40 seconds
Root bark	Light orange	Violent jumping, gasping, loss of equilibrium, lying on lateral side and death at 2 hours 48 minutes.	Same as in the first experiment. Death at 2 hours 45 minutes.	Same as in the first experiment. Death at 2 hours 50 minutes.	2 hours 47 minutes 40 seconds

TABLE - No. 8

TOXICITY OF WATER EXTRACT OF MANGROVES SPECIES TO FINGER LINGS OF CHANOS CHANOS

Name & Part of Mangroves species	Colour when PBS Solution of water extract is added to acclimatised water	Reaction of the Fish			Mean time for lethality
		Expt. 1st	Expt. IInd	Expt. IIIrd	
1) Acanthus ilicifolius					
Seeds	Pale yellow	Vigorous swimming, loss of equilibrium, lying on lateral side, swimming upside down and death at 4 hours 48 minutes.	Same as in the first experiment. Death at 4 hours 1 minute.	Same as in the first experiment. Death at 4 hours 45 minutes.	4 hours 31 minutes 20 seconds
Flowers	Dark yellow	Almost normal.	Normal.	Normal.	-
Leaves	Light yellow	Same as in the experiment with the water extract of A. ilicifolius flowers.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Light yellow	Violent escaping behaviour, vigorous gasping, restless upto 1 hour 48 minutes, then resting, rising to surface, lying on lateral side and death at 4 hours 35 minutes.	Same as in the first experiment. Death at 5 hours 5 minutes.	Same as in the first experiment. Death at 5 hours.	4 hours 53 minutes 20 seconds
2) Acrostichum aureum					
Leaves	Light green	Almost normal behaviour except restless during first 1 hour 45 minutes, then continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Greenish yellow	Normal.	Normal.	Normal.	-

.....2.

3) Avicennia officinalis					
Flowers	Dark green	Almost normal behaviour except restless during first 55 minutes.	Same as in the first experiment.	Same as in the first experiment.	-
Leaves	Green	Same as in the experiment with the water extract of A. officinalis flowers.	Same as in the first experiment.	Same as in the first experiment.	-
Stem	Pale yellow	Almost normal behaviour except at first 1 hour 20 minutes and then continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
4) A. officinalis (Dwarf)					
Leaves	Green	Same as in the experiment with the water extract of A. officinalis leaves.	Same as in the first experiment.	Same as in the first experiment.	-
5) Brugguiera cylindrica					
Seeds	Orange	Violent escaping behaviour, jumping, gasping, loss of equilibrium, gulping, lying on lateral side and upside down movements. Death at 4 hours 3 minutes.	Slight escaping behaviour, loss of equilibrium, lying on lateral side and slight jumping. Death at 4 hours.	Same as in the first experiment. Death at 4 hours 1 minute.	4 hours 1 minute 20 seconds
Leaves	Dark orange	Almost same as in the experiment with the water extract of B. cylindrica seeds. Death at 4 hours 5 minutes.	Same as in the first experiment. Death at 4 hours 10 minutes.	Same as in the first experiment. Death at 4 hours 15 minutes.	4 hours 10 minutes
Stem bark	Orange	Same as in the experiment with water extract of B. cylindrica seeds. Death at 3 hours 30 minutes.	Same as in the first experiment. Death at 3 hours 10 minutes.	Same as in the first experiment. Death at 3 hours 40 minutes.	3 hours 26 minutes 40 seconds

.....3.

6) Clerodendrum inerme					
Flowers	Yellowish green	Vigorous jumping, restless, loss of equilibrium, swimming upside down, lying on lateral side and death at 4 hours 20 minutes.	Same as in the first experiment. Death at 4 hours 30 minutes.	Same as in the first experiment. Death at 4 hours.	4 hours 16 minutes 40 seconds
Leaves	Dark green	Same as in the experiment with the water extract of C. inerme flower (Above) Death at 4 hours 30 minutes.	Same as in the first experiment. Death at 4 hours 20 minutes.	Same as in the first experiment. Death at 4 hours 45 minutes.	4 hours 31 minutes 40 seconds
Stem	Yellowish green	Normal.	Normal.	Normal.	-
7) Excoccaria agallocha					
Leaves	Orange	Violent escaping behaviour, gasping, loss of equilibrium, swimming upside down and death at 1 hour 10 minutes.	Same as in the first experiment. Death at 1 hour 25 minutes.	Same as in the first experiment. Death at 1 hour 5 minutes.	1 hour 13 minutes 20 seconds
Stem bark	Pale yellow	Same as in the experiment with water extract of E. agallocha leaves. Death at 1 hour 5 minutes.	Same as in the first experiment. Death at 1 hour 4 minutes.	Same as in the first experiment. Death at 1 hour 10 minutes.	1 hour 6 minutes 20 seconds
8) Rhizophora mucronata					
Seeds	Orange	Violent swimming, escaping behaviour, restless and loss of equilibrium. Death at 3 hours 5 minutes.	Same as in the first experiment. Death at 3 hours.	Slow swimming, resting and then restless loss of equilibrium and death at 3 hours 20 minutes.	3 hours 8 minutes 20 seconds
Leaves	Grayish	Almost normal behaviour except at times restless during the first 1 hour 5 minutes and later continued normal.	Same as in the first experiment.	Same as in the first experiment.	-
Stem bark	Dark orange	Same as in the experiment with water extract of R. mucronata seeds. Death at 4 hours 20 minutes.	Same as in the first experiment. Death at 4 hours 10 minutes.	Same as in the first experiment. Death at 4 hours 8 minutes.	4 hours 12 minutes 40 seconds
Root bark	Orange	Same as in the experiment with the water extract of R. mucronata seeds. Death at 4 hours 40 minutes.	Same as in the first experiment. Death at 4 hours 30 minutes.	Same as in the first experiment. Death at 4 hours 48 minutes.	4 hours 39 minutes 20 seconds

- = No lethality was observed.

TABLE - 9a

LETHALITY AND NON-LETHALITY OF FISHES TO THE MANGROVE EXTRACTS

ETHANOL EXTRACTS

Name and Parts of Mangrove species	Liza macrolepis	Tilapia mossambica	Chanos chanos
1. Acanthus ilicifolius			
Seeds	-	-	+
Flowers	-	-	-
Leaves	-	-	-
Stem	-	-	+
2. Acrostichum aureum			
Leaves	-	+	-
Stem	-	-	-
3. Avicennia officinalis			
Flowers	-	-	-
Leaves	-	-	-
Stem	-	-	-
4. A. officinalis (Dwarf)			
Leaves	-	-	-
5. Bruguiera cylindrica			
Seeds	-	-	+
Leaves	-	-	+
Stem bark	+	+	+
6. Clerodendrum inerme			
Flowers	+	-	+
Leaves	-	-	+
Stem	-	-	-
7. Excoccaria agallocha			
Leaves	+	+	+
Stem bark	+	+	+
8. Rhizophora mucronata			
Seeds	+	+	+
Leaves	+	+	-
Stem bark	+	+	+
Root bark	+	+	+

TABLE - 9b

WATER EXTRACT

Name and Part of mangrove species	Liza macrolepis	Tilapia mossambica	Chanos chanos
1. Acanthus ilicifolius			
Seeds	-	-	+
Flowers	-	-	-
Leaves	-	-	-
Stem	-	-	+
2. Acrostichum aureum			
Leaves	-	+	-
Stem	-	-	-
3. Avicennia officinalis			
Flowers	-	-	-
Leaves	-	-	-
Stem	-	-	-
4. A. officinalis (Dwarf)			
Leaves	-	-	-
5. Bruguiera cylindrica			
Seeds	-	-	+
Leaves	-	-	+
Stem bark	+	+	+
6. Clerodendrum inerme			
Flowers	+	-	+
Leaves	-	-	+
Stem	-	-	-
7. Excoccaria agallocha			
Leaves	+	+	+
Stem bark	+	+	+
8. Rhizophora mucronata			
Seeds	+	+	+
Leaves	+	+	-
Stem bark	+	+	+
Root bark	+	+	+

(+) Lethality was observed
(-) No lethality was observed.

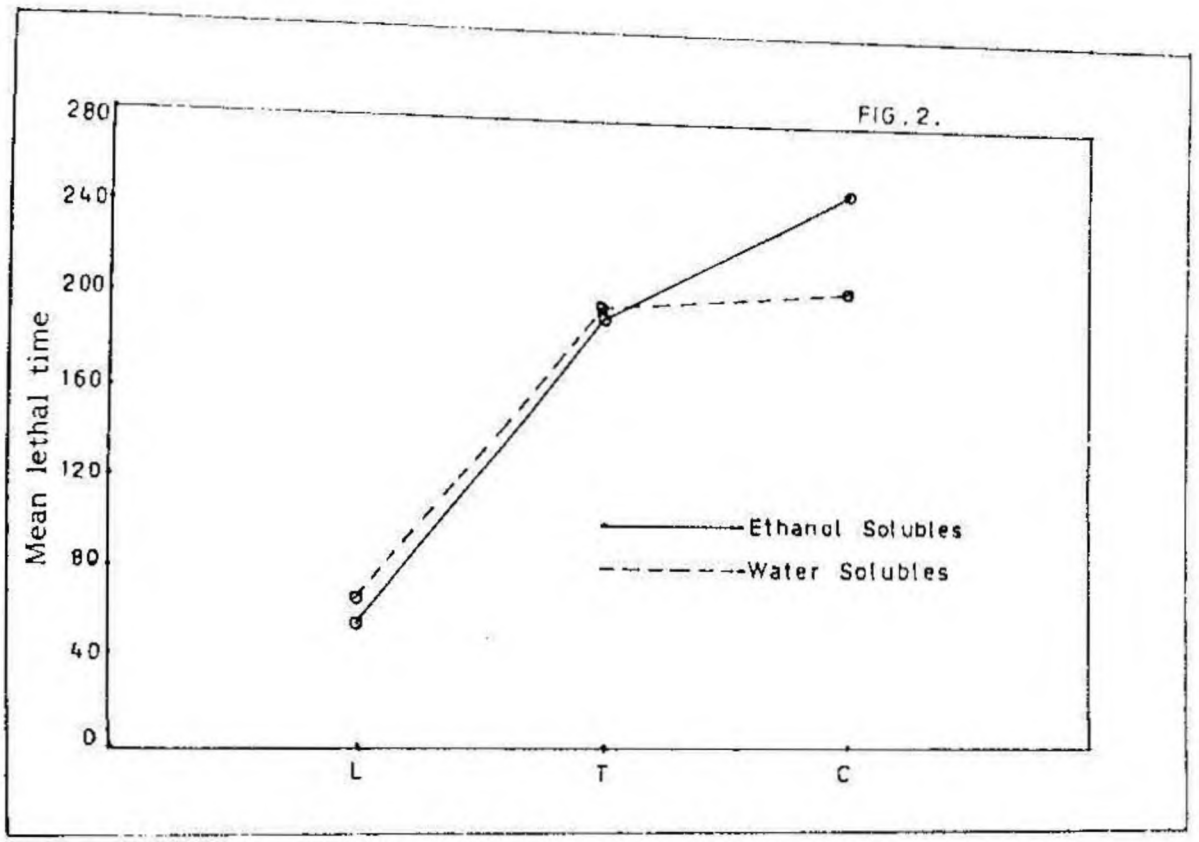

Fig.2. The time taken for lethality of fishes to the ethanol and water solubles of Stem bark of B. cylindrica

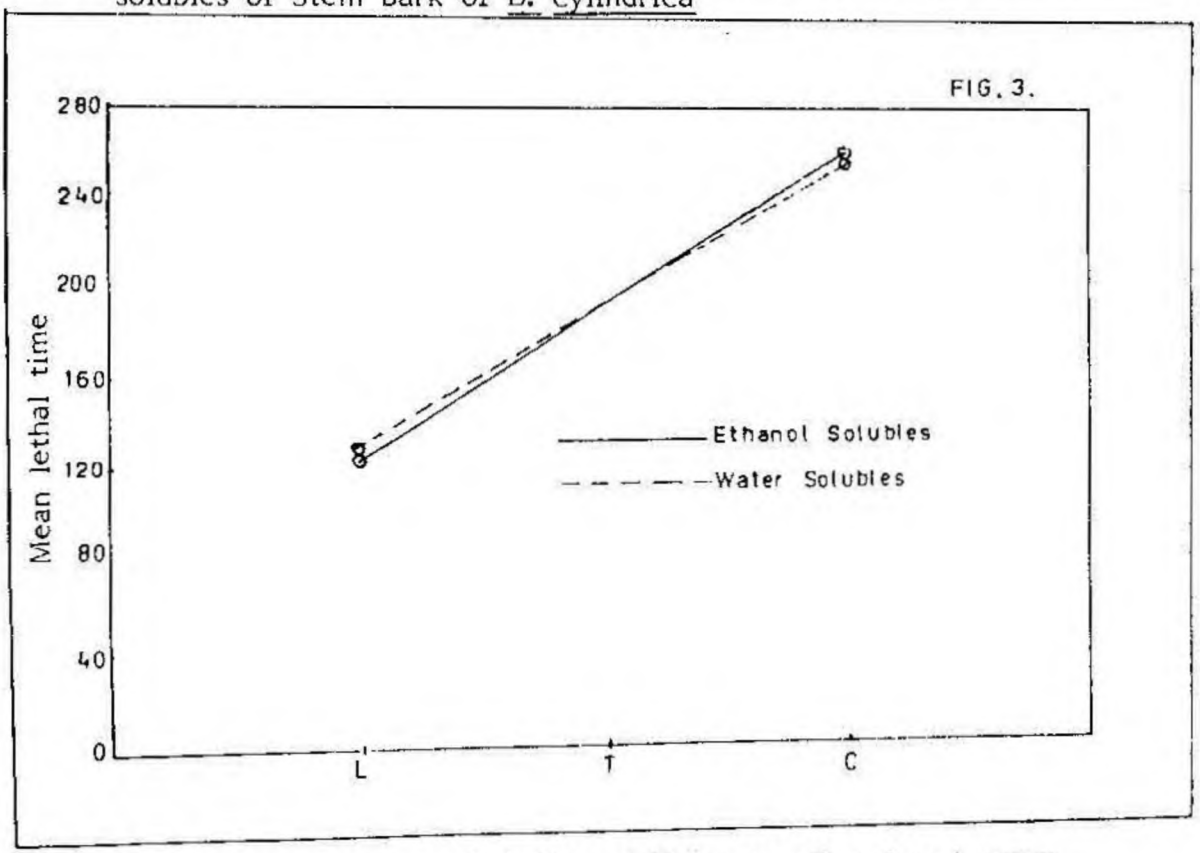

Fig.3. The time taken for lethality of fishes to ethanol and water solubles of flower of C. inerme
L - Liza macrolepis; T - Tilapia mossambica; C - Chanos chanos

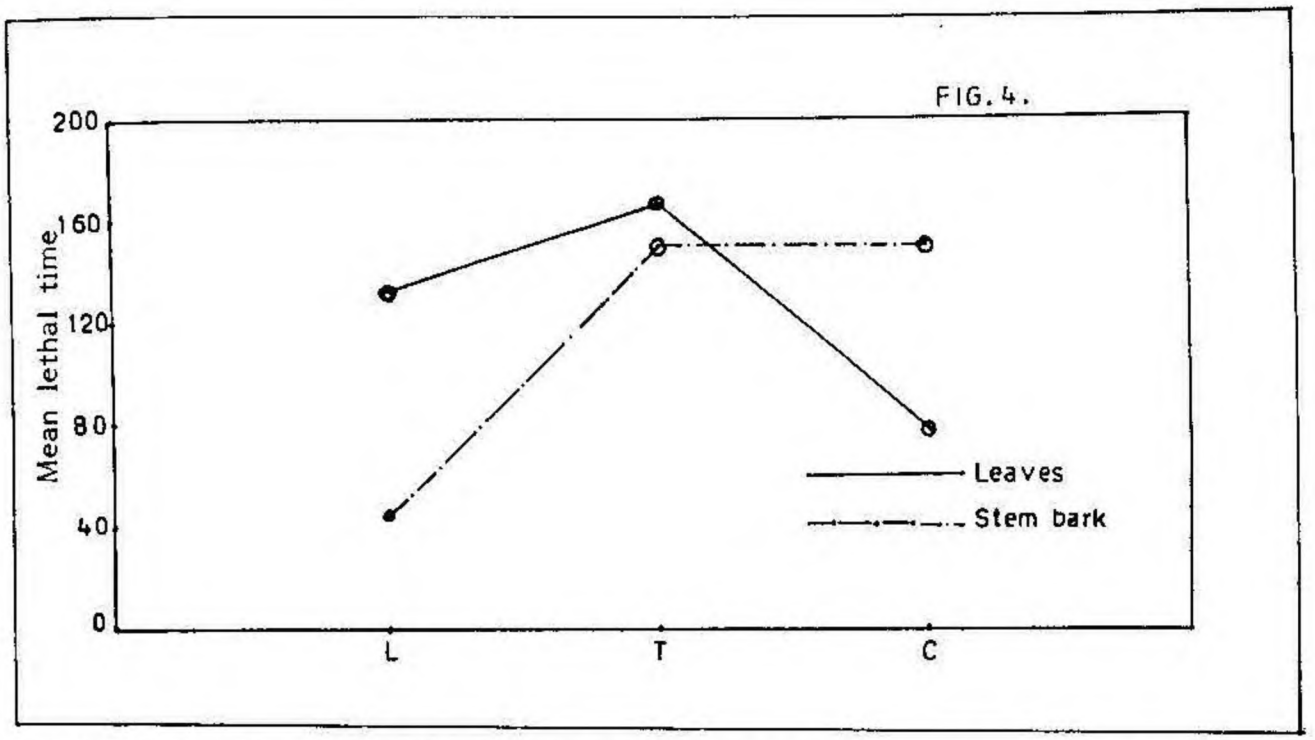

Fig.4. The time taken for lethality of fishes to the ethanol solubles of E. agallocha

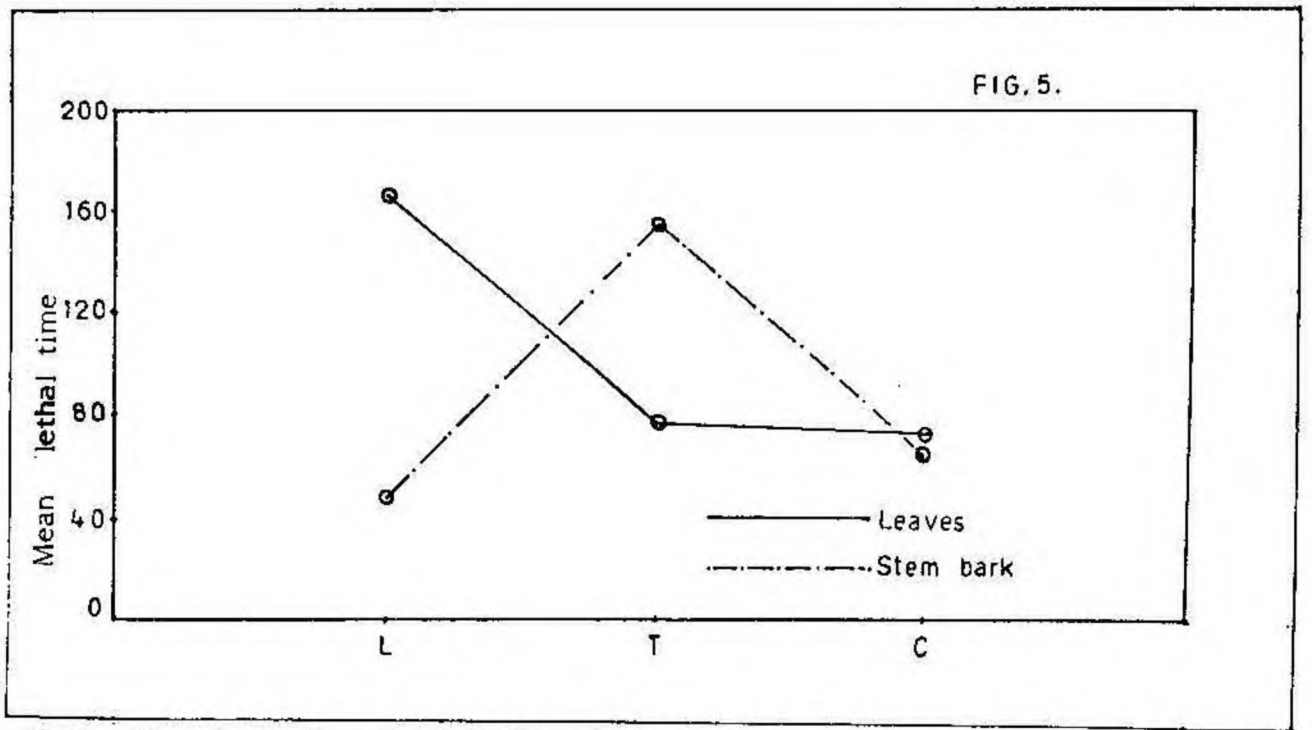

Fig.5. The time taken for lethality of fishes to the water solubles of E. agallocha L - Liza macrolepis; T - Tilapia mossambica - C - Chanos chanos

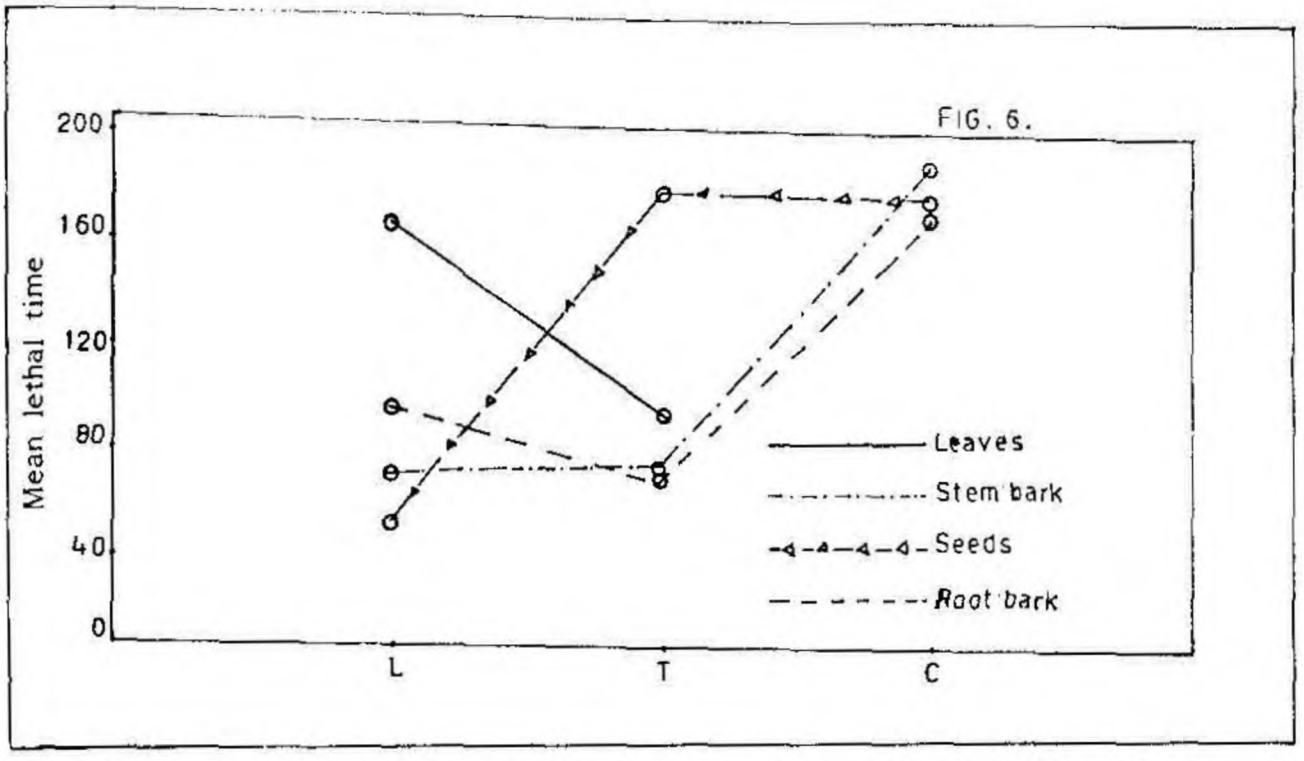

Fig. 6. The time taken for lethality of fishes to the ethanol solubles of R. mucronata

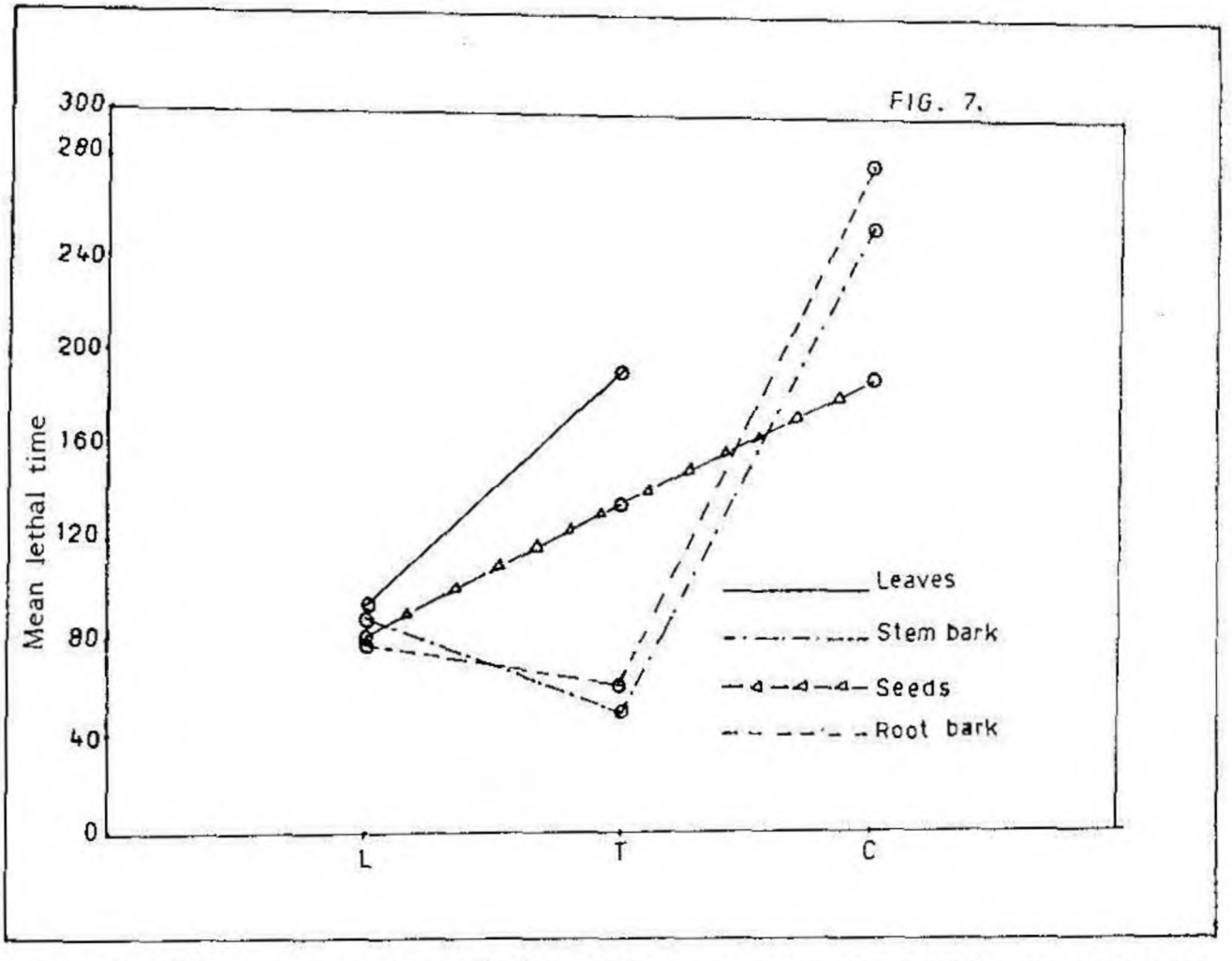

Fig. 7. The time taken for lethality of fishes to the water solubles R. mucronata
L - Liza macrolepis; T - Tilapia mossambica; C - Chanos chanos

Explanation of histograms.

AiSd	-	*Acanthus ilicifolius* Seeds.
AiSt	-	*Acanthus ilicifolius* Stem.
AaLe	-	*Acrostichum aureum* Leaves.
BcSd	-	*Bruguiera cylindrica* Seeds.
BcSb	-	*Bruguiera cylindrica* Stem bark.
CiFl	-	*Clerodendrum inerme* Flower.
CiLe	-	*Clerodendrum inerme* Leaves.
EaLe	-	*Excoccaria agallocha* Leaves.
EaSb	-	*Excoccaria agallocha* Stem bark.
RmSd	-	*Rhizophora mucronata* Seeds.
RmLe	-	*Rhizophora mucronata* Leaves.
RmSb	-	*Rhizophora mucronata* Stem bark.
RmRb	-	*Rhizophora mucronata* Root bark.

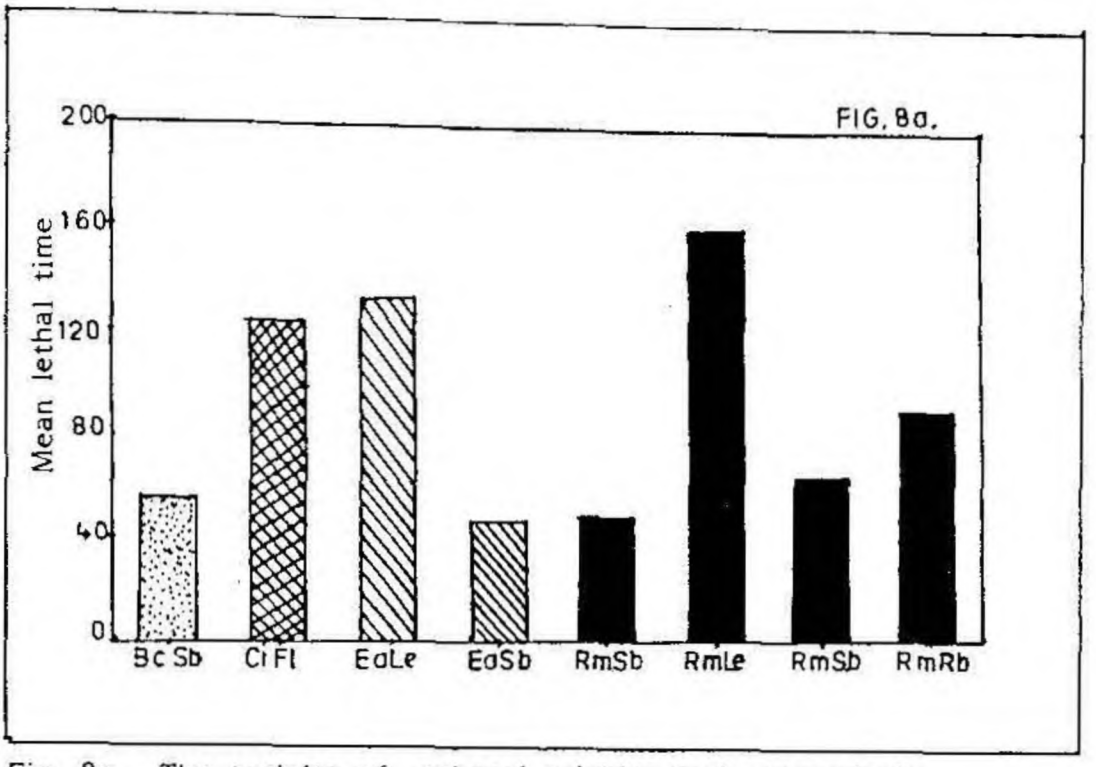

Fig. 8a. The toxicity of ethanol solubles to L. macrolepis

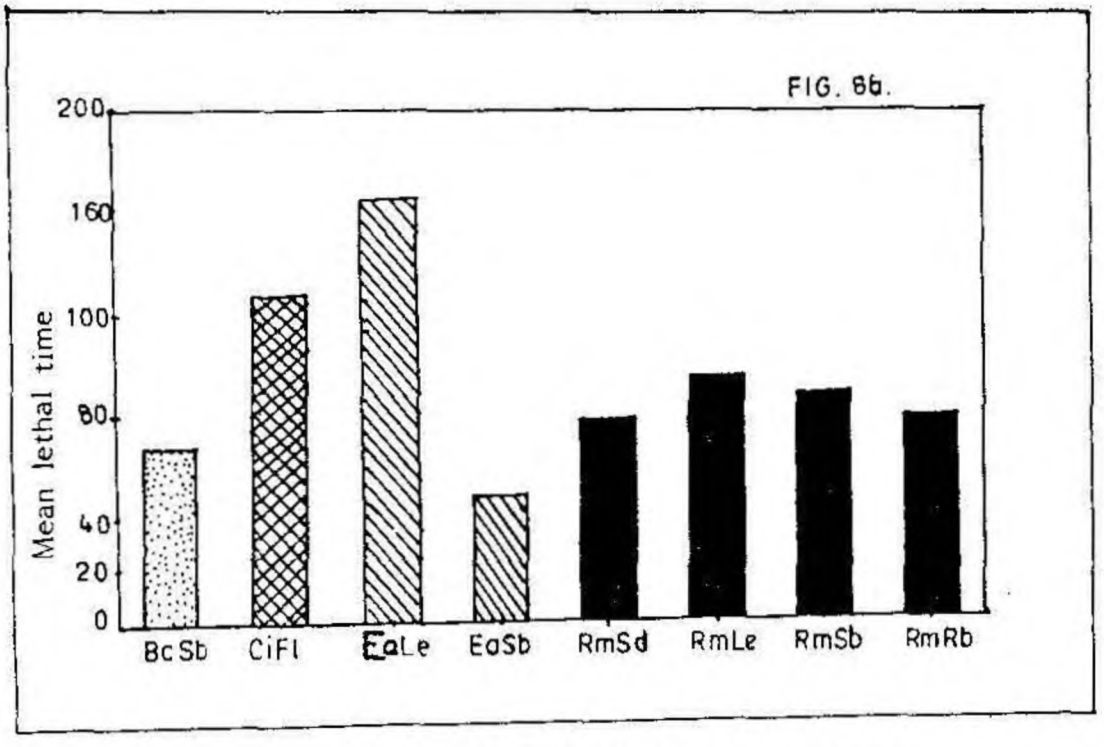

Fig. 8b. The toxicity of water soluble to L. macrolepis

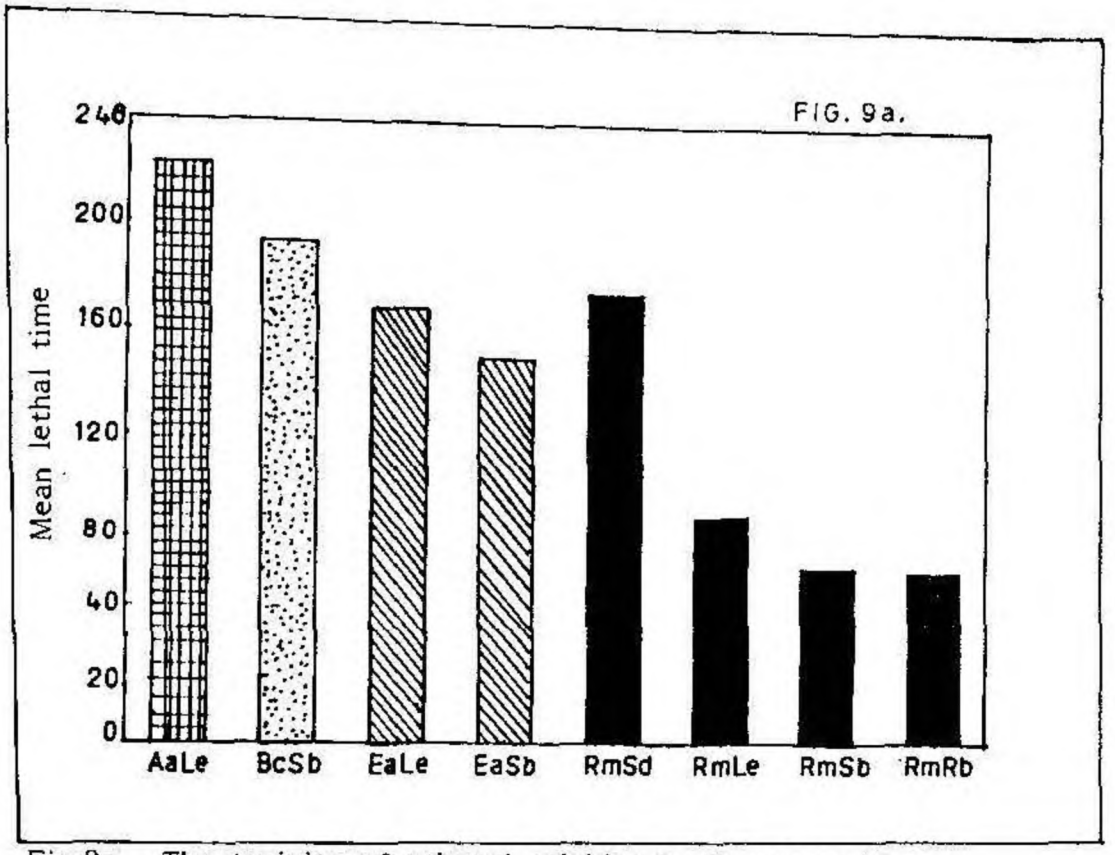

Fig.9a. The toxicity of ethanol solubles to T. mossambica

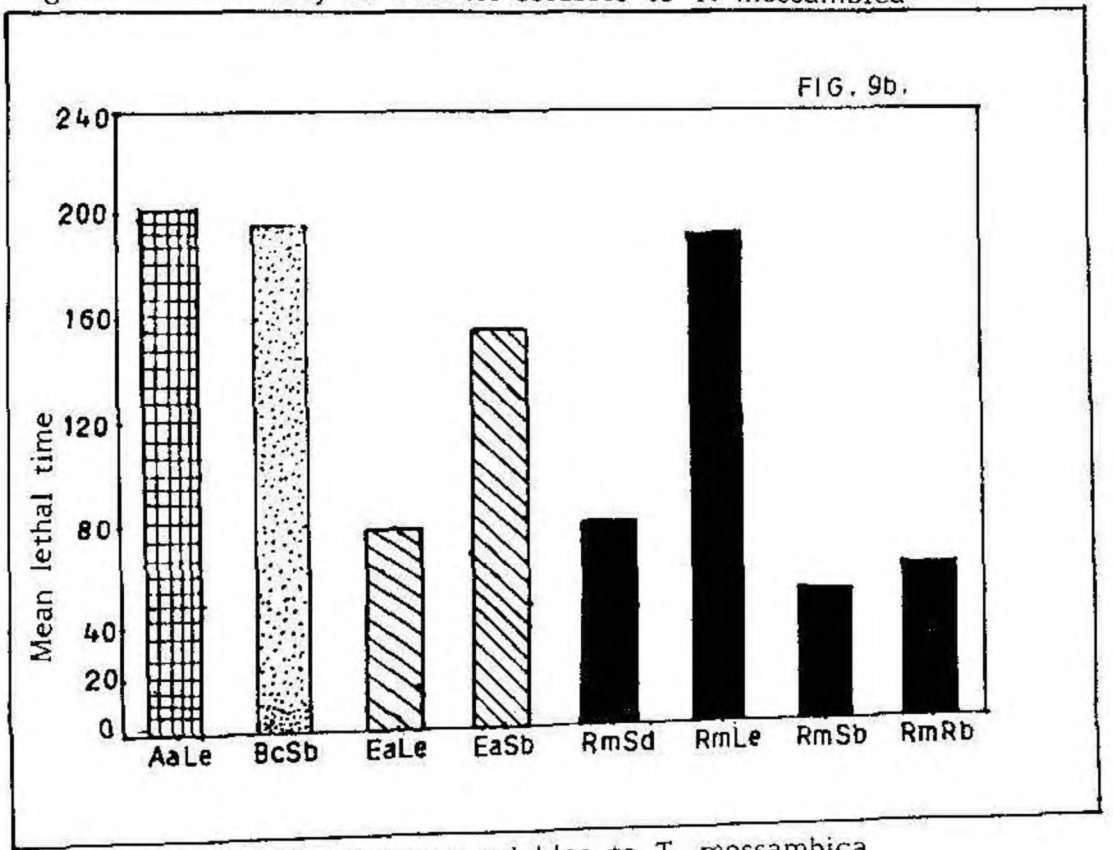

Fig.9b. The toxicity of water solubles to T. mossambica

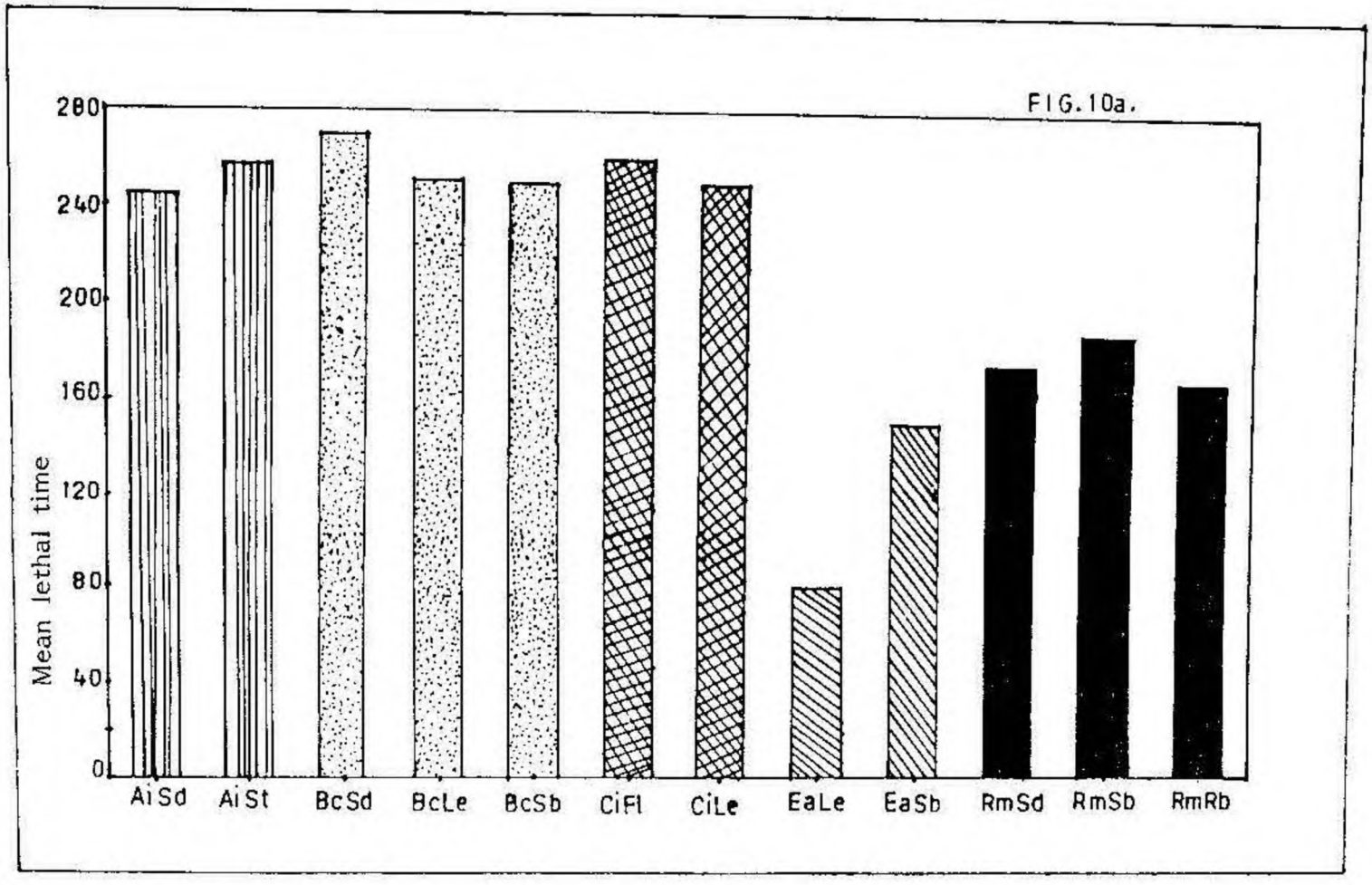

Fig. 10a. Toxicity of ethanol solubles to C. chanos

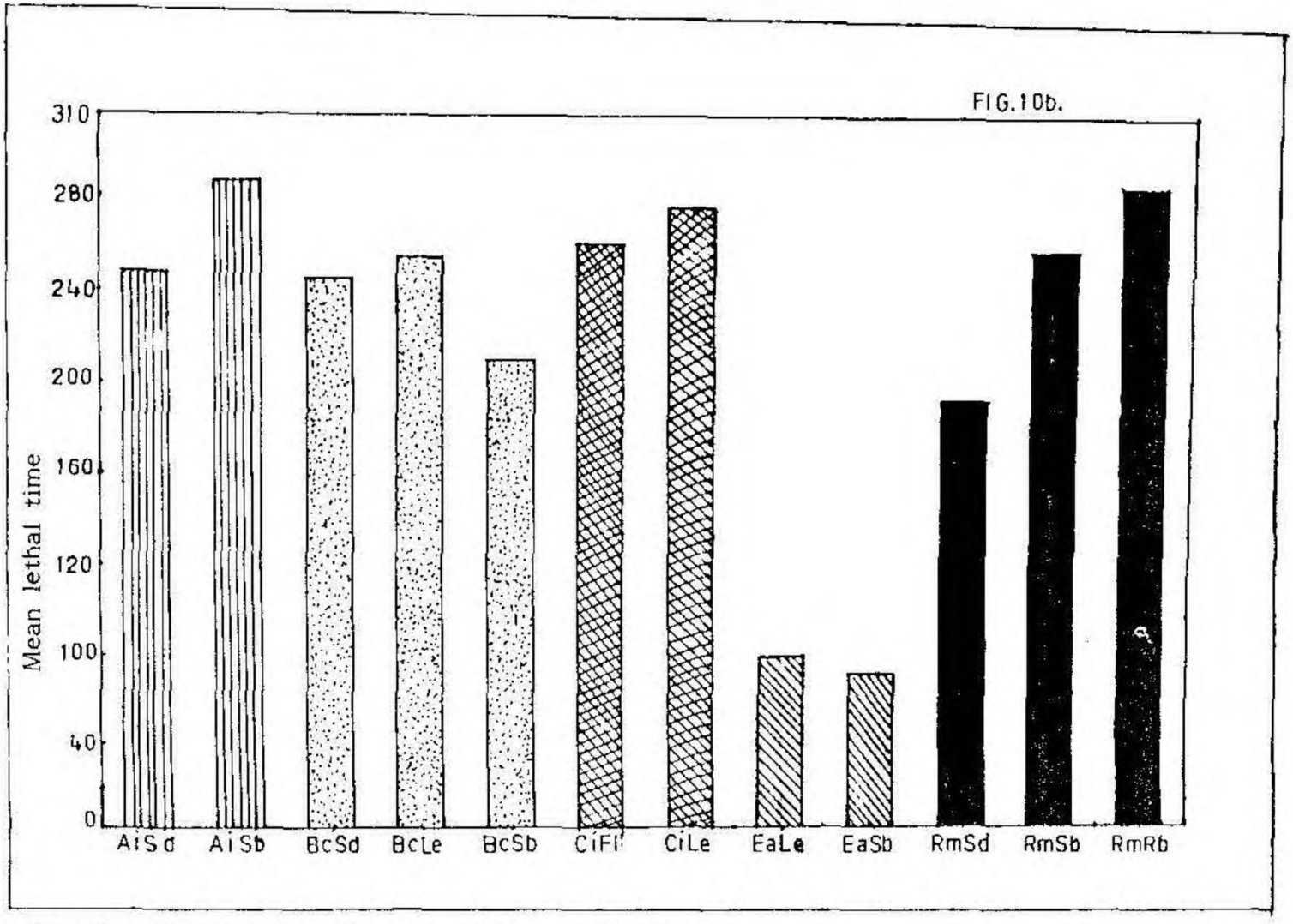

Fig.10b. The toxicity of water solubles to C. chanos

the ethanol and water extracts of stem bark of B. cylindrica, leaves and stem bark of E. agallocha and seeds, stem and root bark of R. mucronata. The ethanol and water solublesof seeds and stem bark of A. ilicifolius were lethal to the fingerlings of C. chanos, leaves of A. aureum to T. mossambica, seeds and leaves of B. cylindrica to C. chanos, flower of C. inerme to L. macrolepis and C. chanos. Both the extract of leaves of C. inerme produced lethality to C. chanos only. Both extract of leaves of R. mucronata produced lethality to L. macrolepis and T. mossambica only.

Tables 10 & 11 gives the total free sugar, total protein and cholesterol content in the muscle tissue of L. macrolepis where lethality was produced by ethanol and water extracts of mangrove species along with the values of the control fishes. Tables 12 & 13 give the percentage of total free sugar, total protein and cholesterol in the muscle tissue of T. mossambica whereas tables 14 and 15 show the contents of these in the muscle tissue of C. chanos after lethality was observed in both the extracts. In all cases the total free sugar, total protein and cholesterol were less than those of the control fish.

Table 16 gives the Rf values of the different spots observed in the paper chromatography in five solvent systems when the ethanol extracts of each part of mangrove species is paper chromatographed.

TABLE - 10

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF LIZA MACROLEPIS FROM BIOTOXICITY ASSAYS WITH ETHANOLIC EXTRACTS.

Name and parts of mangrove species		Mean value of Biochemical analysis		
		Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1.	Control	0.4850	17.825	0.5045
2.	Bruguiera cylindrica - Stem bark	0.1312	12.829	0.2485
3.	Clerodendrum inerme - Flowers	0.1062	16.125	0.3850
4.	Excoccaria agallocha - Leaves	0.0976	14.825	0.468
5.	E. agallocha - Stem bark	0.0887	11.926	0.425
6.	Rhizophora mucronata - Seeds	0.0484	11.485	0.465
7.	R. mucronata - Leaves	0.0725	12.330	0.285
8.	R. mucronata - Stem bark	0.0825	10.625	0.368
9.	R. mucronata - Root bark	0.0982	11.445	0.295

TABLE - 11

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF LIZA MACROLEPIS FROM BIOTOXICITY ASSAYS WITH WATER EXTRACTS.

Name and parts of Mangrove species		Mean value of Biochemical analysis		
		Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1.	Control	0.4182	17.0125	0.5265
2.	Bruguiera cylindrica - Stem bark	0.0921	11.925	0.3010
3.	Clerodendrum inerme - Flowers	0.0625	11.625	0.4110
4.	Excoccaria agallocha - Leaves	0.0920	13.998	0.4280
5.	E. agallocha - Stem bark	0.0625	11.825	0.385
6.	Rhizophora mucronata - Seeds	0.1025	12.835	0.325
7.	R. mucronata - Leaves	0.0762	12.440	0.308
8.	R. mucronata - Stem bark	0.0825	11.025	0.465
9.	R. mucronata - Root bark	0.0925	11.925	0.311

TABLE - 12

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF TILAPIA MOSSAMBICA FROM BIOTOXICITY ASSAYS WITH ETHANOLIC EXTRACTS.

Name and parts of mangrove species		Mean value of biochemical analysis		
		Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1.	Control	0.8135	20.825	0.4226
2.	Acrostichum aureum - Leaves	0.4826	17.763	0.2325
3.	Bruguiera cylindrica - Stem bark	0.356	16.823	0.1668
4.	Excoccaria agallocha - Leaves	0.3862	13.628	0.1105
5.	E. agallocha - Stem bark	0.395	13.842	0.1540
6.	Rhizophora mucronata - Seeds	0.3208	14.825	0.2283
7.	R. mucronata - Leaves	0.1743	17.625	0.1637
8.	R. mucronata - Stem bark	0.5666	12.115	0.2517
9.	R. mucronata - Root bark	0.4383	13.343	0.2153

TABLE - 13

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF TILAPIA MOSSAMBICA FROM BIOTOXICITY ASSAYS WITH WATER EXTRACTS

Name and parts of mangrove species		Mean value of biochemical analysis		
		Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1.	Control	0.8732	20.982	0.4935
2.	Acrostichum aureum - Leaves	0.3125	18.732	0.2025
3.	Bruguiera cylindrica - Stem bark	0.3563	16.363	0.1079
4.	Excoccaria agallocha - Leaves	0.3862	12.163	0.2862
5.	E. agallocha - Stem bark	0.3851	13.432	0.1385
6.	Rhizophora mucronata - Seeds	0.3208	15.826	0.3432
7.	R. mucronata - Leaves	0.2621	12.825	0.3925
8.	R. mucronata - Stem bark	0.4868	17.6282	0.3468
9.	R. mucronata - Root bark	0.4396	13.825	0.2360

TABLE - 14

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF CHANOS CHANOS FROM BIOTOXICITY ASSAYS WITH ETHANOLIC EXTRACTS

Name and parts of mangrove species	Mean value of biochemical analysis		
	Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1. Control	0.9881	19.685	0.6251
2. Acanthus ilicifolius - Seeds	0.6626	17.480	0.5258
3. A. ilicifolius - Stem	0.7810	17.895	0.4251
4. Bruguiera cylindrica - Seeds	0.5196	16.160	0.2135
5. B. cylindrica - Stem bark	0.6715	16.825	0.2930
6. Clerodendrum inerme - Flowers	0.4160	15.123	0.3212
7. C. inerme - Leaves	0.7860	15.925	0.3895
8. Excoccaria agallocha - Leaves	0.1654	16.025	0.4212
9. E. agallocha - Stem bark	0.2352	15.989	0.4825
10. Rhizophora mucronata - Seeds	0.8250	14.212	0.3215
11. R. mucronata - Stem bark	0.6320	15.012	0.3925
12. R. mucronata - Root bark	0.5420	15.112	0.3012

TABLE - 15

TOTAL FREE SUGAR, TOTAL PROTEIN AND CHOLESTEROL CONTENT IN THE MUSCLE TISSUE OF CHANOS CHANOS FROM BIOTOXICITY ASSAYS WITH THE WATER EXTRACTS

Name and part of mangrove species		Mean value of biochemical analysis		
		Total free sugar (mg%)	Total protein (mg%)	Cholesterol (mg%)
1.	Content	0.9213	19.786	0.6135
2.	Acanthus ilicifolius - Seeds	0.5262	16.840	0.4820
3.	A. ilicifolius - Stem	0.6280	18.482	0.3212
4.	Bruguiera cylindrica - Seeds	0.4960	17.872	0.5921
5.	B. cylindrica - Stem bark	0.5715	16.284	0.5410
6.	Clerodendrum inerme - Flowers	0.4168	15.925	0.4312
7.	C. inerme - Leaves	0.6789	16.834	0.2212
8.	Excoccaria agallocha - Leaves	0.2183	16.125	0.3121
9.	E. agallocha - Stem bark	0.222	15.695	0.3980
10.	Rhizophora mucronata - Seeds	0.625	13.211	0.4825
11.	R. mucronata - Stem bark	0.723	14.112	0.3212
12.	R. mucronata - Stem bark	0.543	14.985	0.3920

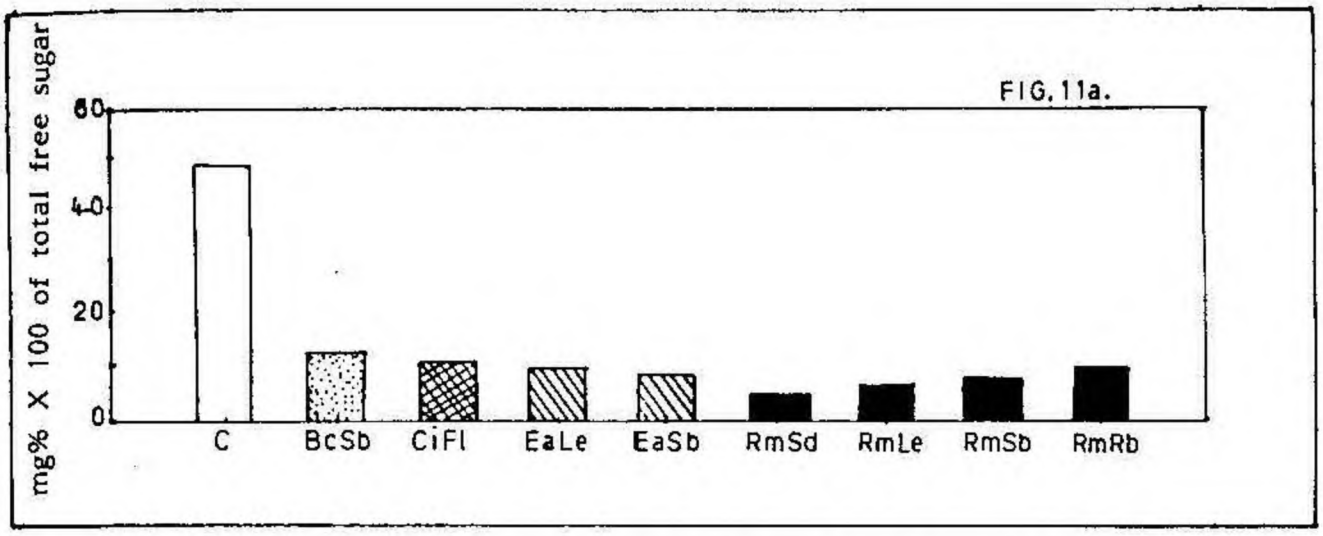

Fig.11a. The total free sugar in muscle tissue of L. macrolepis(ethanol solubles)

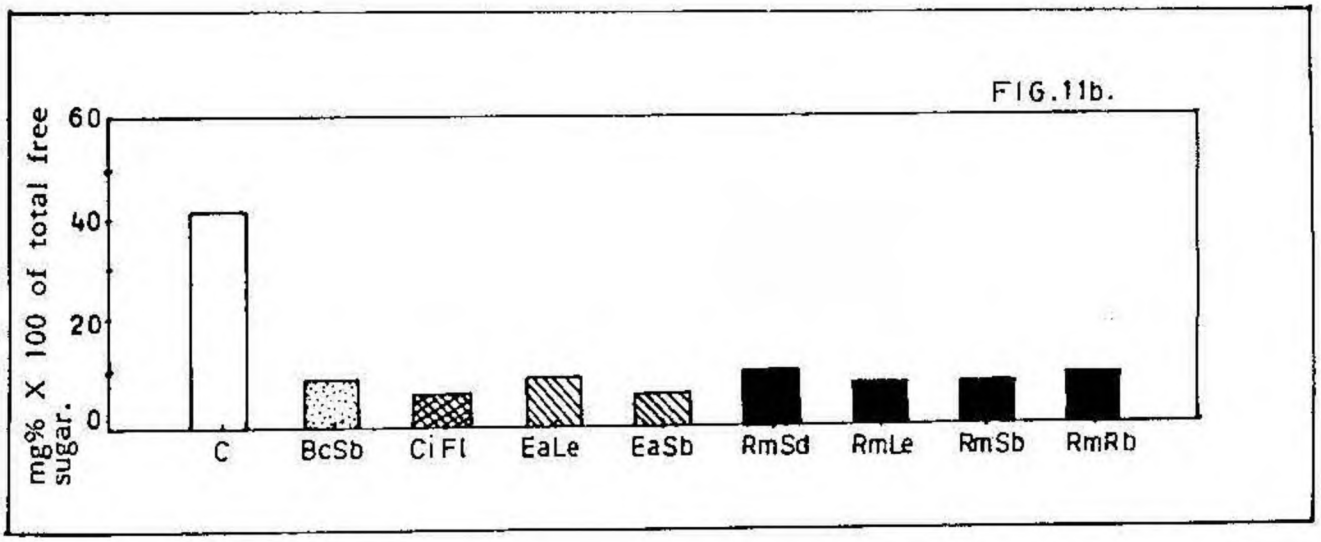

Fig.11b. The total free sugar in muscle tissue of L. macrolepis(water solubles)

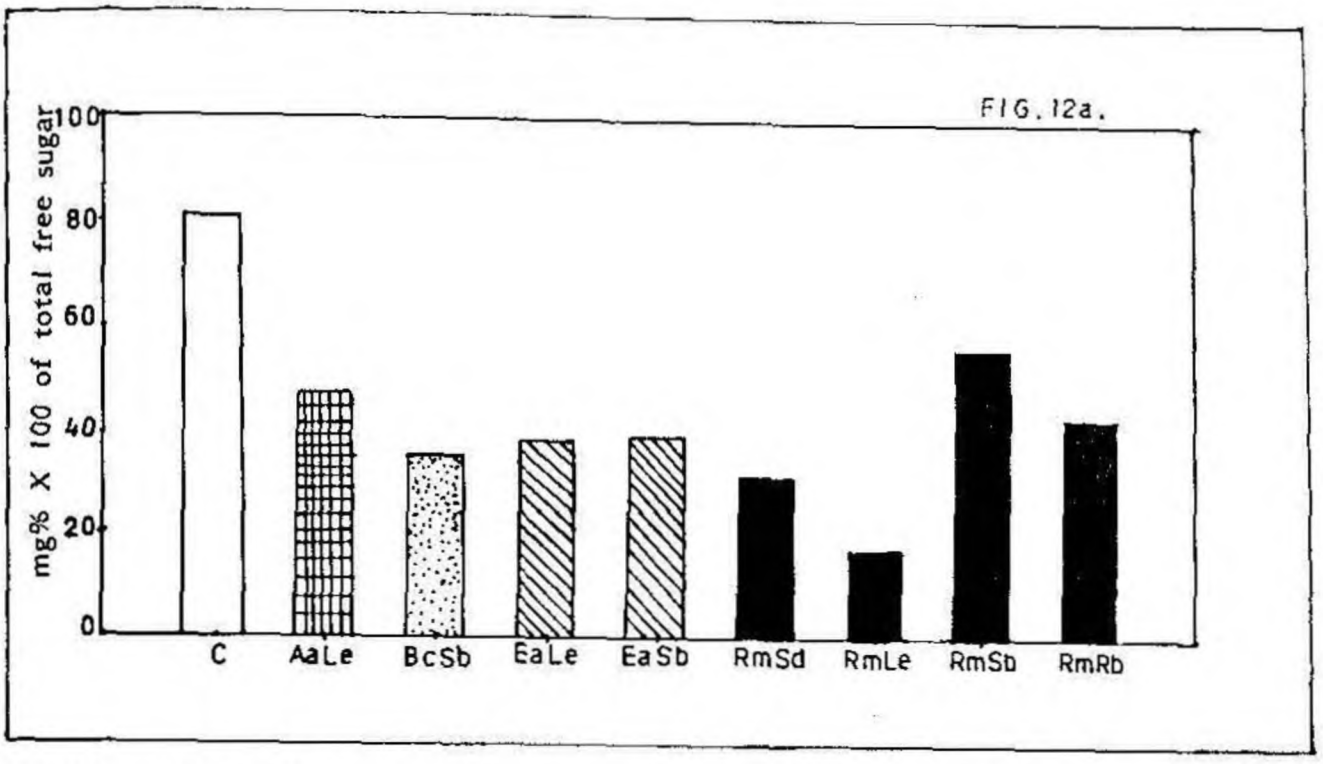

Fig.12a. The total free sugar in muscle tissue of T. mossambica (ethanol soluble)

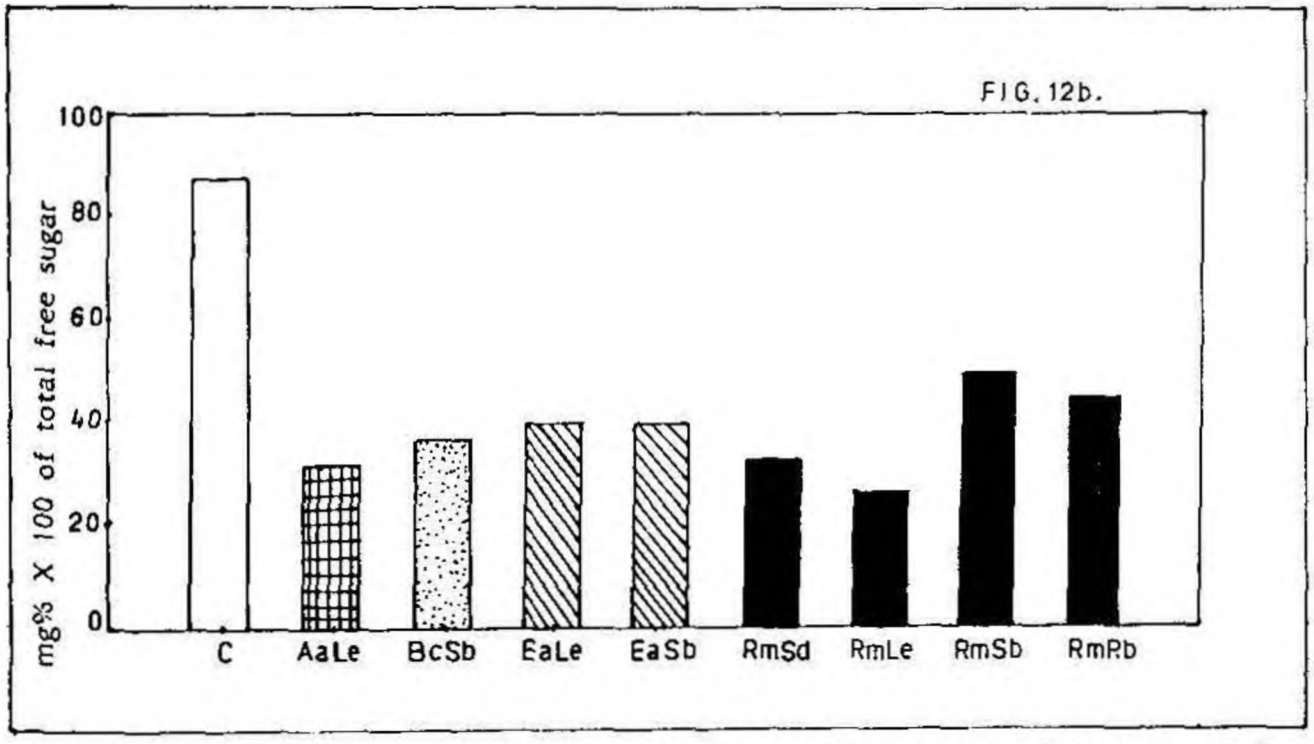

Fig.12b. The total free sugar of muscle tissue of T. mossambica (Water solubles)

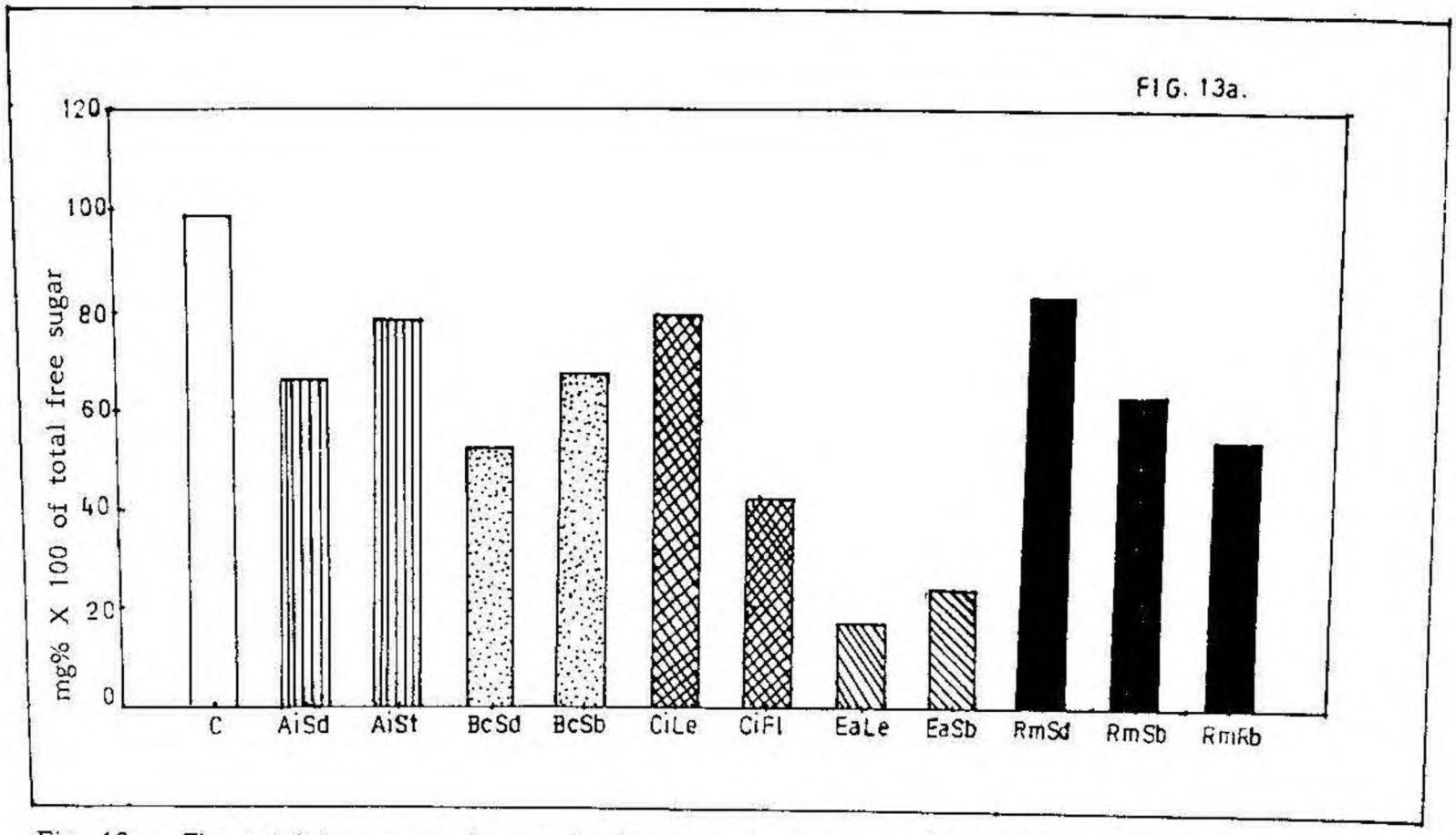

Fig. 13a. The total free sugar in muscle tissue of C. chanos (ethanol soluble)

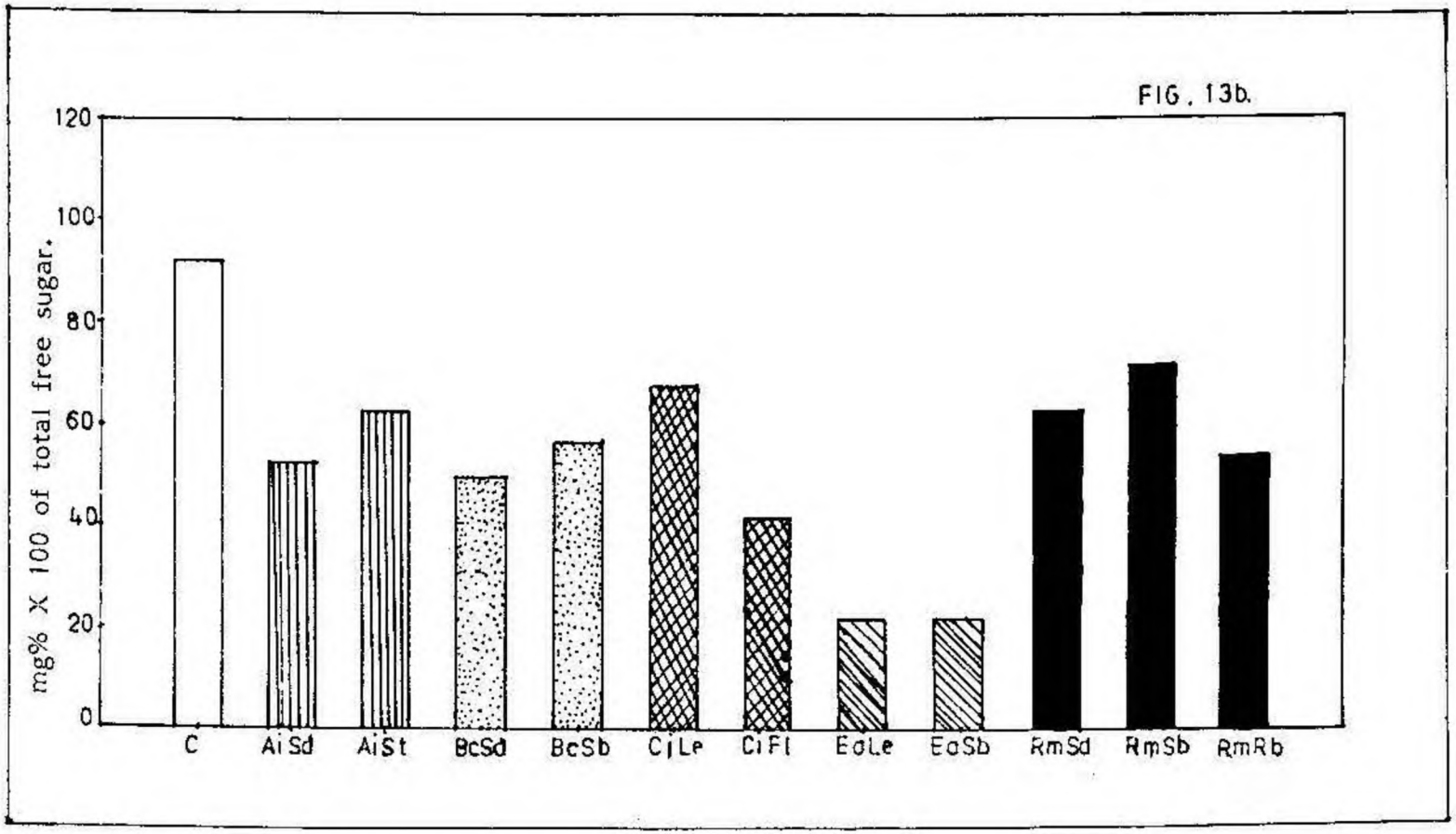

Fig. 13b. The total free sugar is muscle tissue of C. chanos (water soluble)

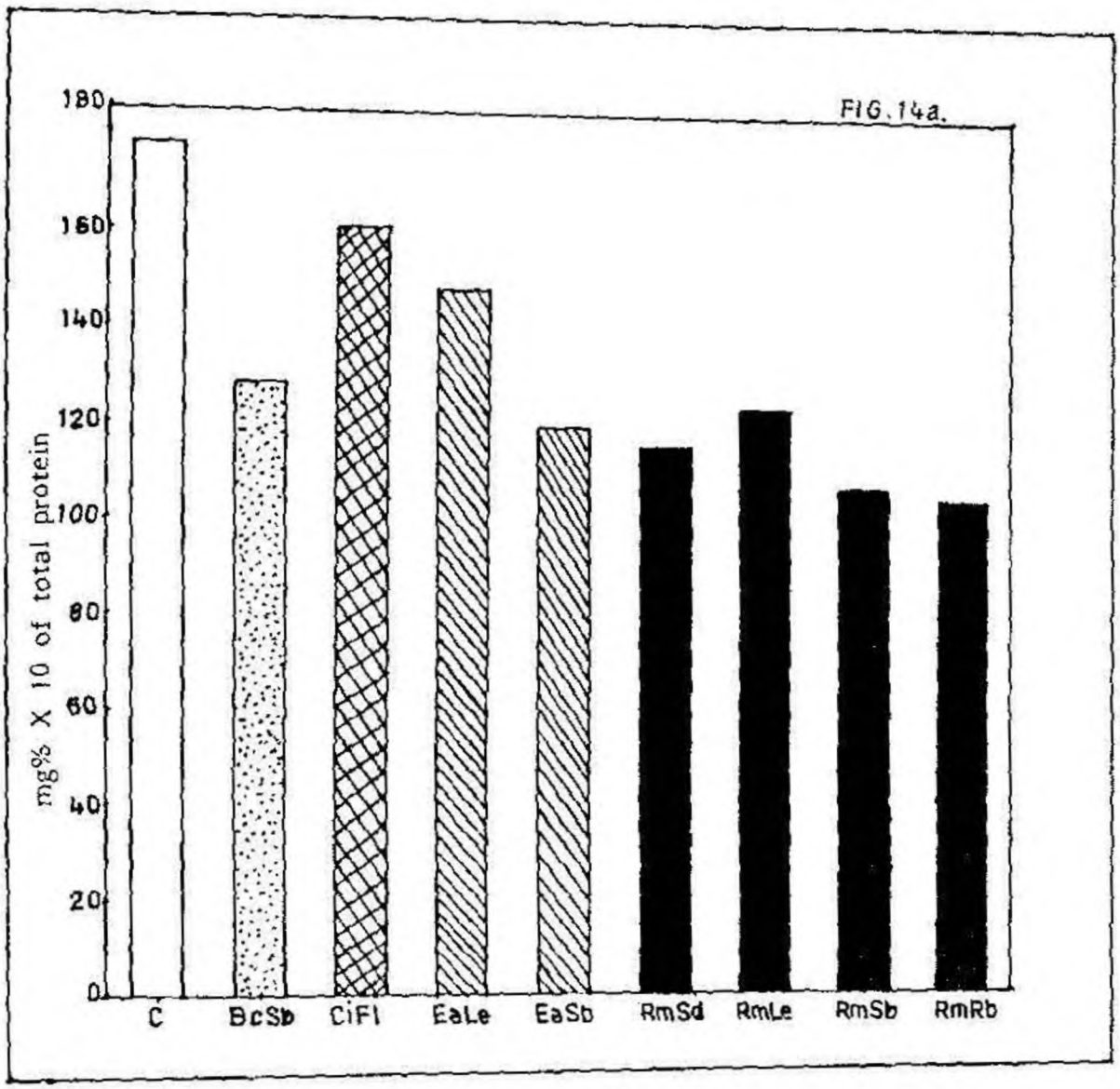

Fig. 14a. *The total protein in muscle tissue of* L. mucrolepis *(ethanol soluble)*

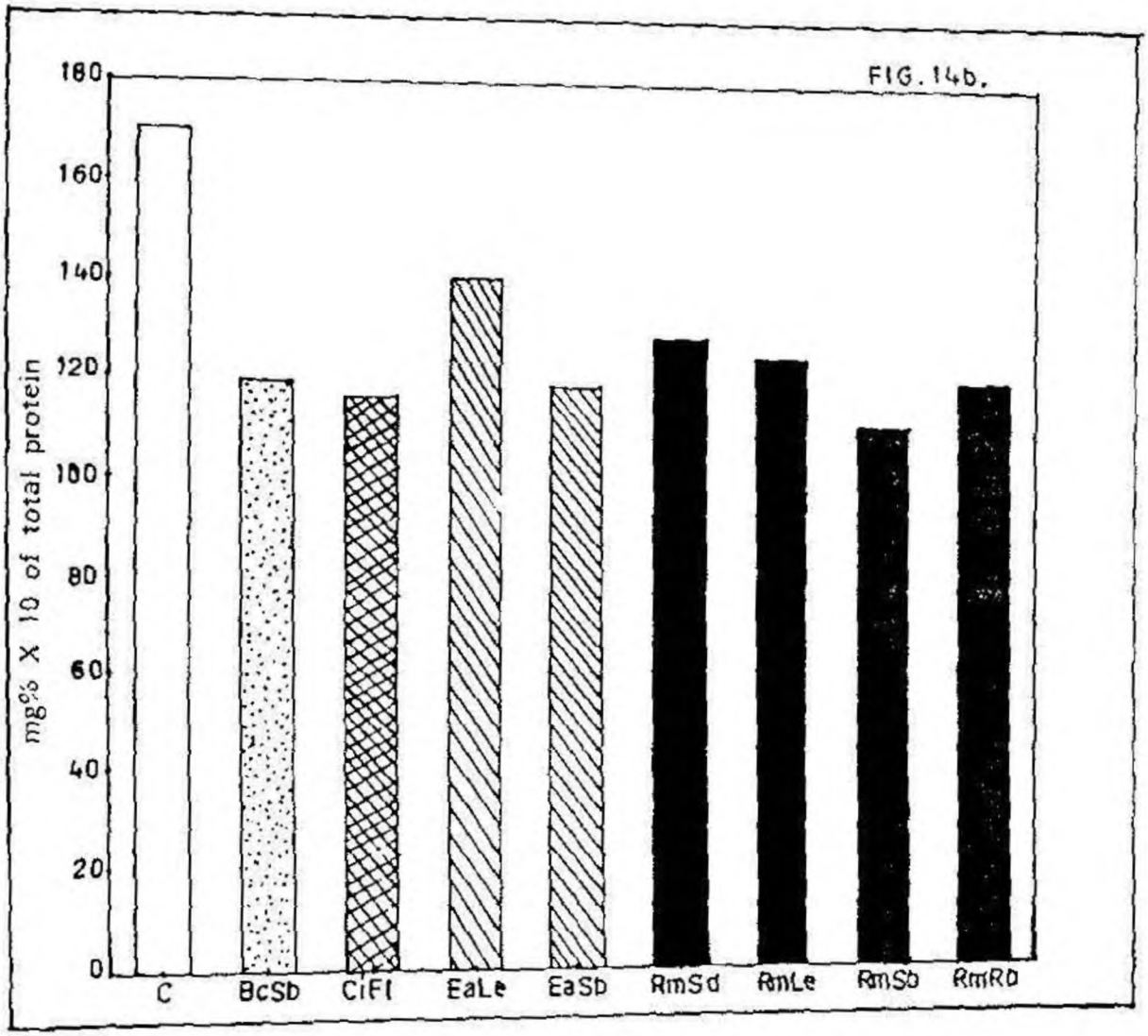

Fig. 14b. The total protein in muscle tissue of L. macrolepis (water soluble)

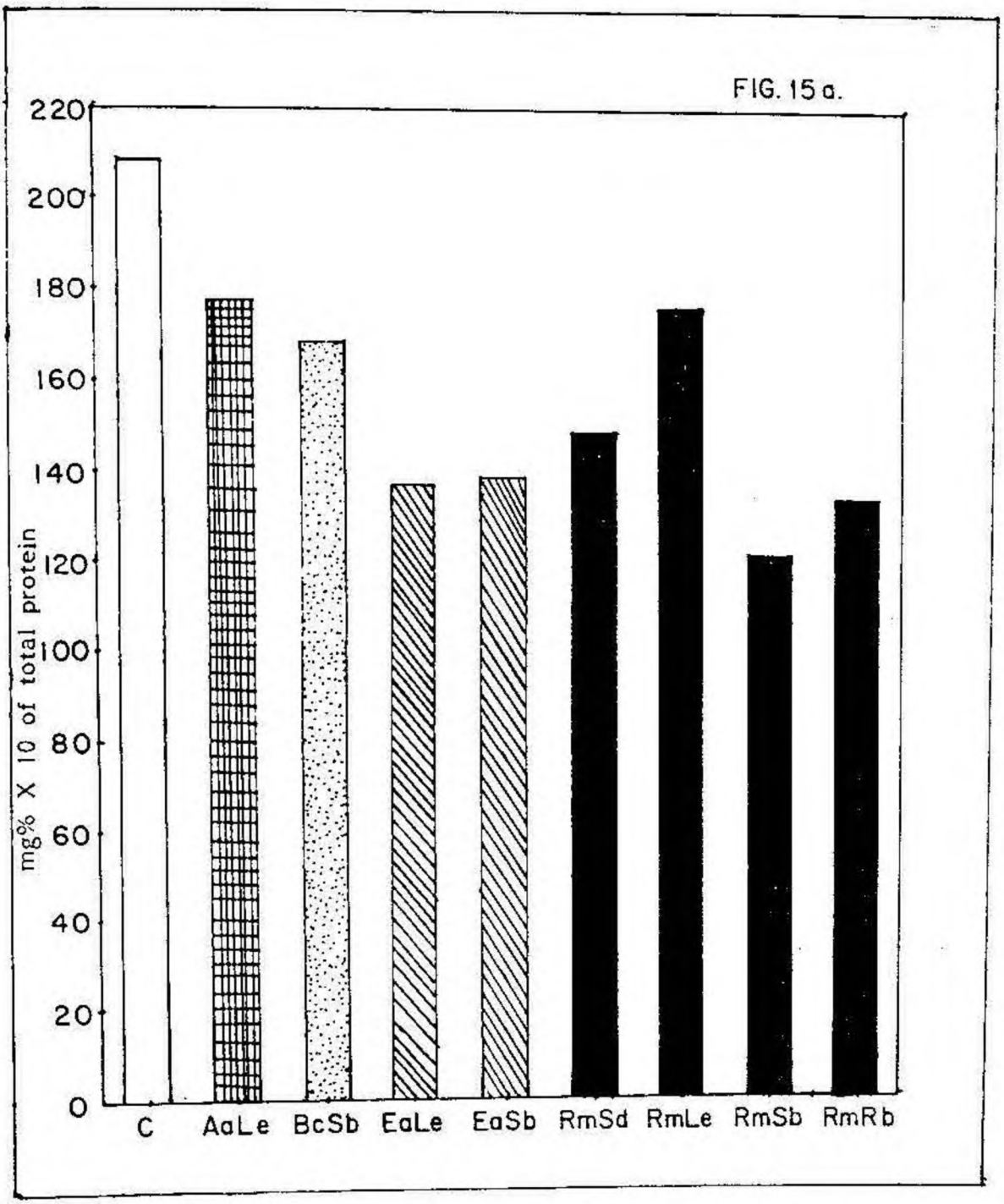

Fig.15a. The total protein in muscle tissue of T. mossambica (ethanol soluble)

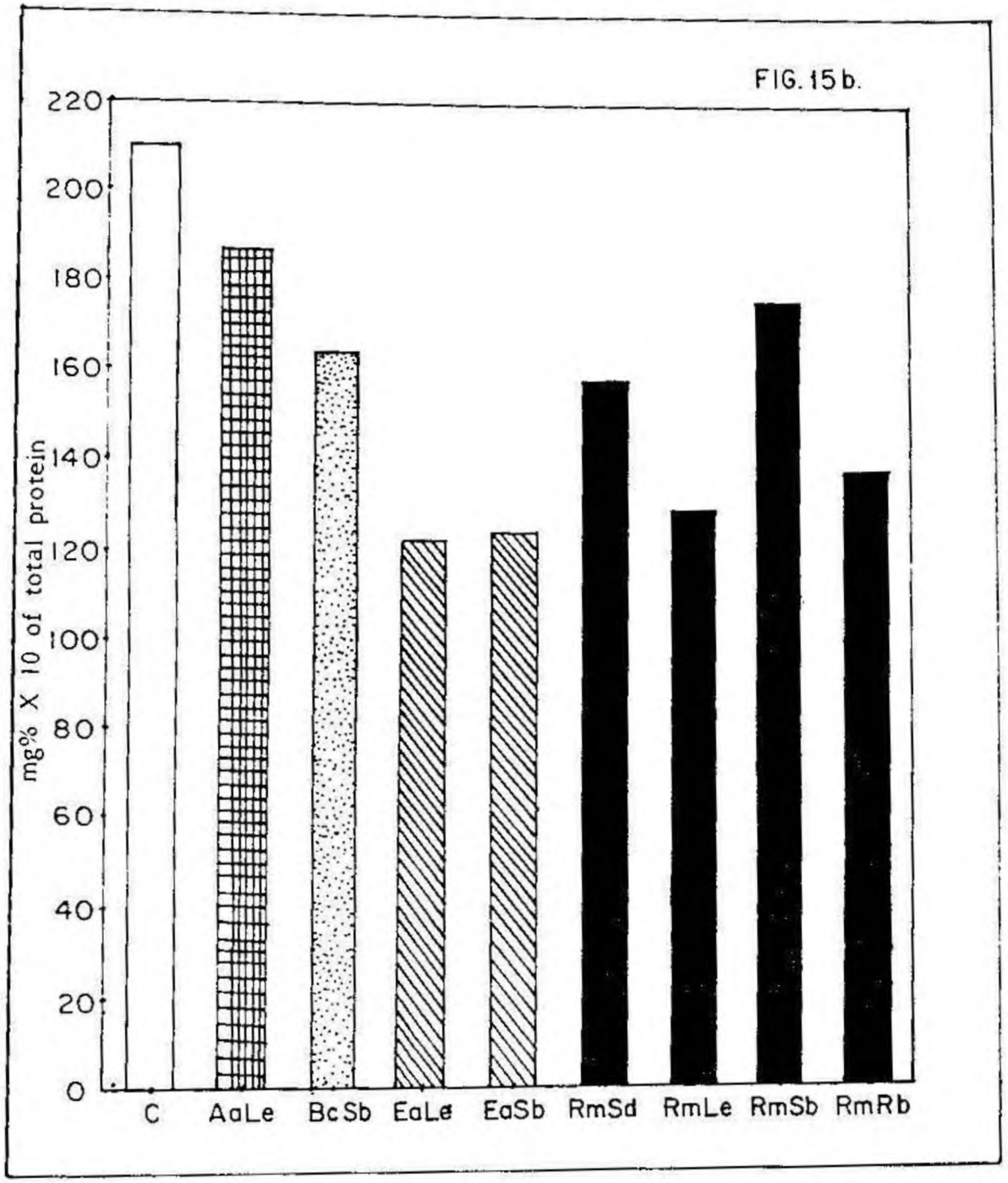

Fig.15b. The total protein in muscle tissue of T. mossambica (water solubles)

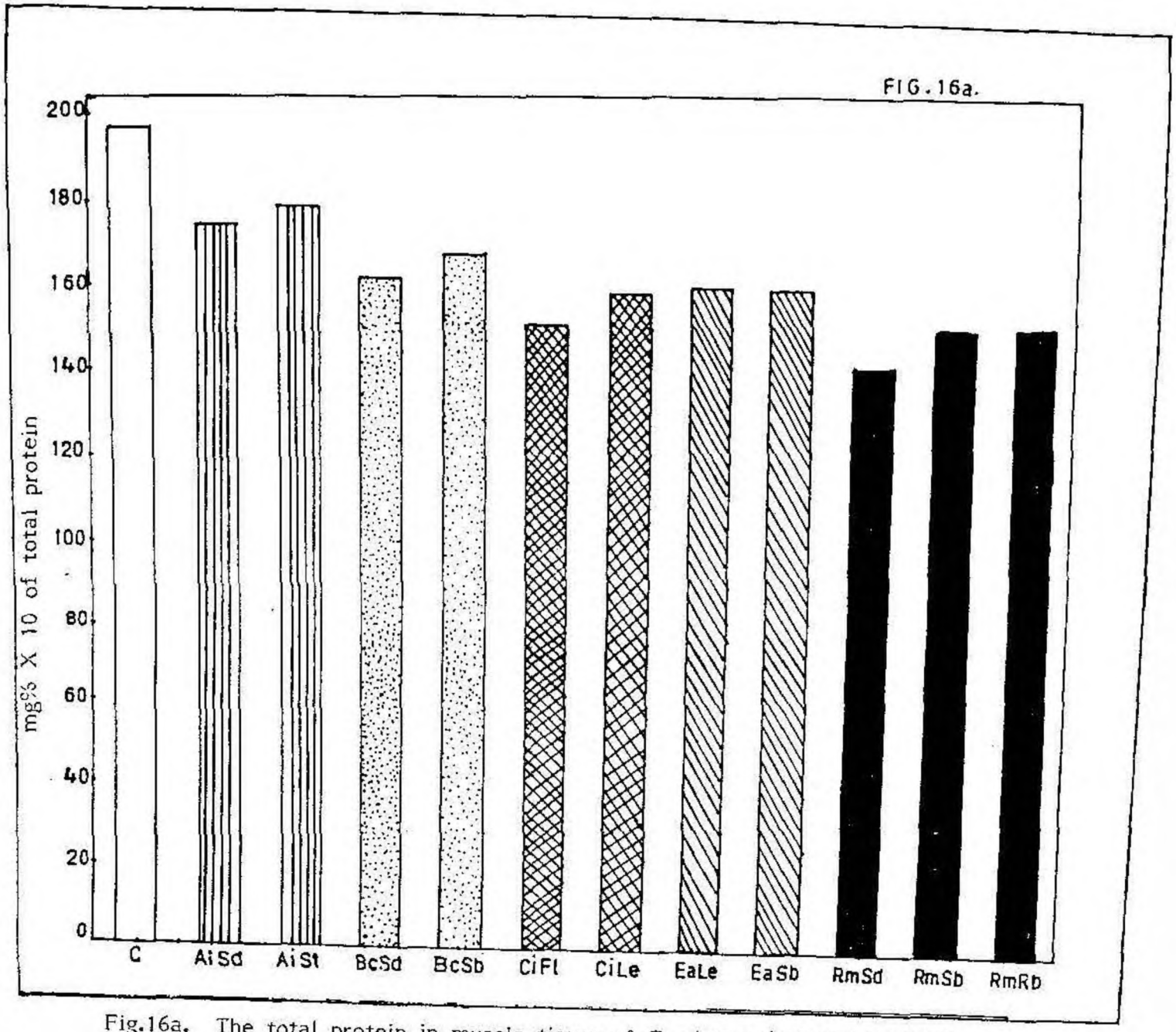

Fig.16a. The total protein in muscle tissue of C. chanos (ethanol soluble)

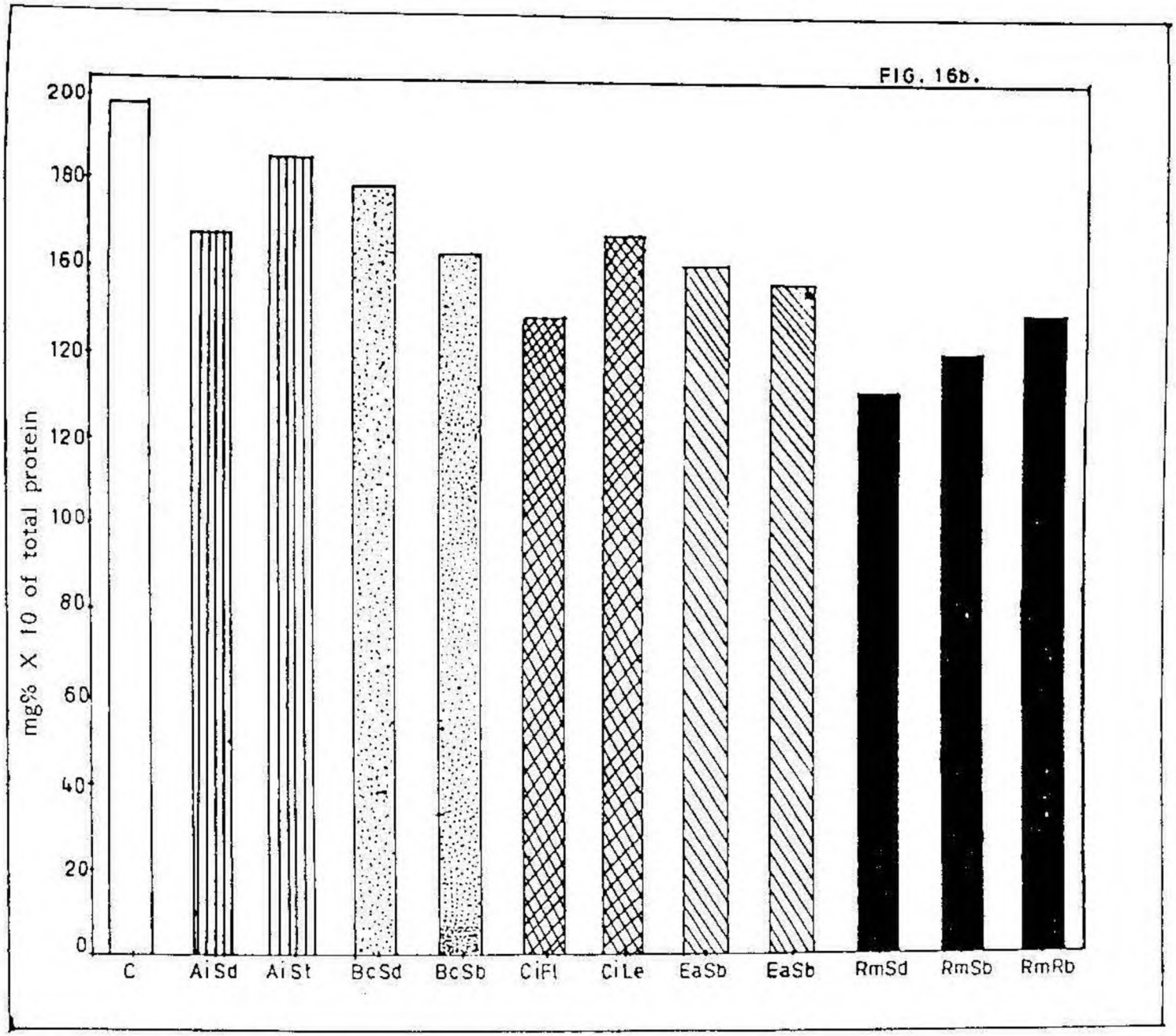

Fig.16b. The total protein in muscle tissue of C. chanos (water solubles)

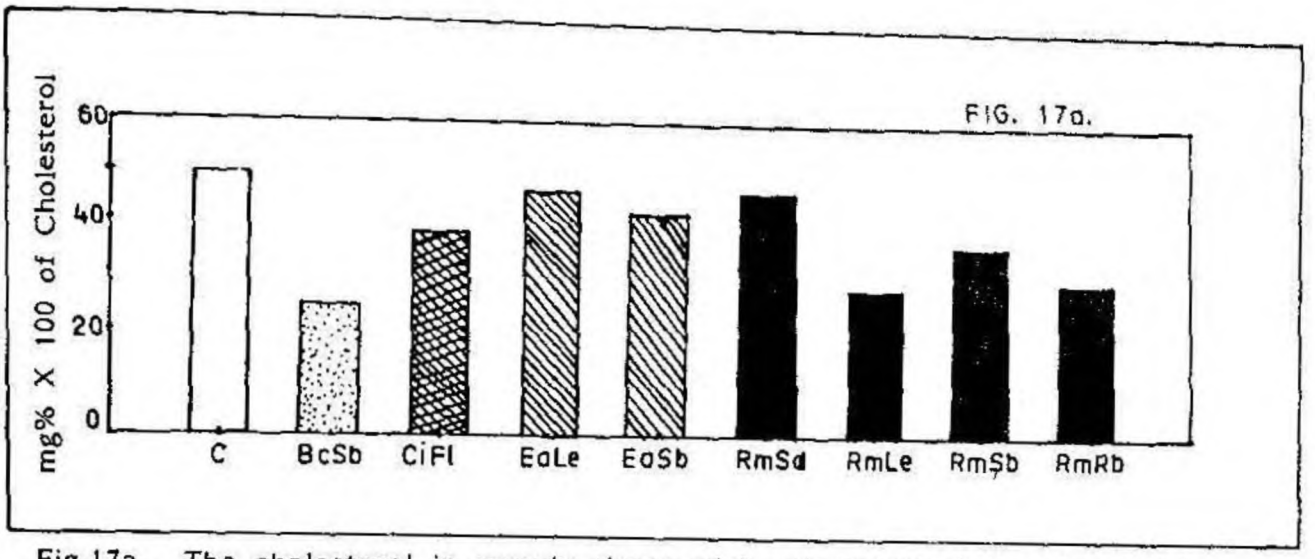

Fig.17a. The cholesterol in muscle tissue of L. macrolepis (ethanol soluble)

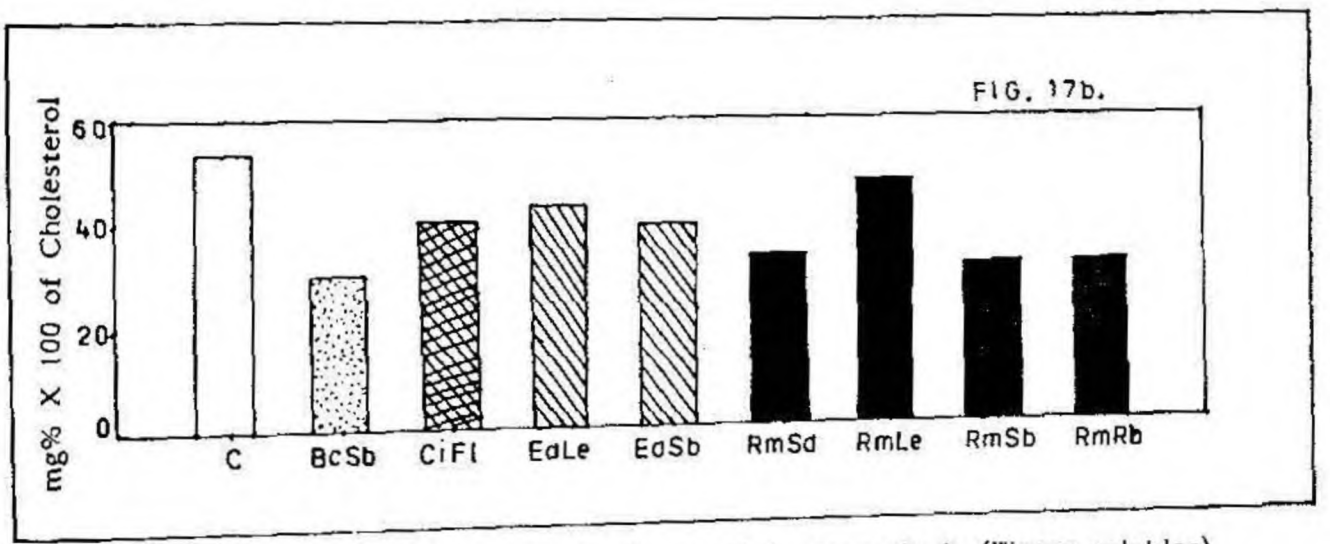

Fig. 17b. The cholesterol in muscle tissue of L. macrolepis (Water solubles)

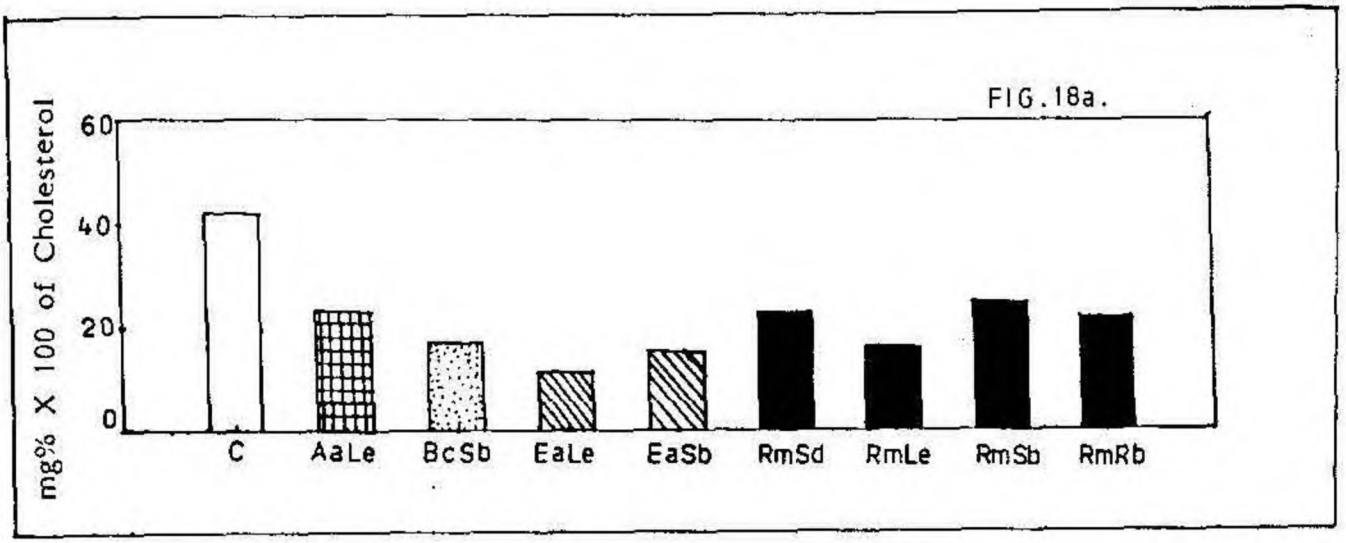

Fig.18a. The cholesterol in muscle tissue of T. mossambica (ethanol solubles)

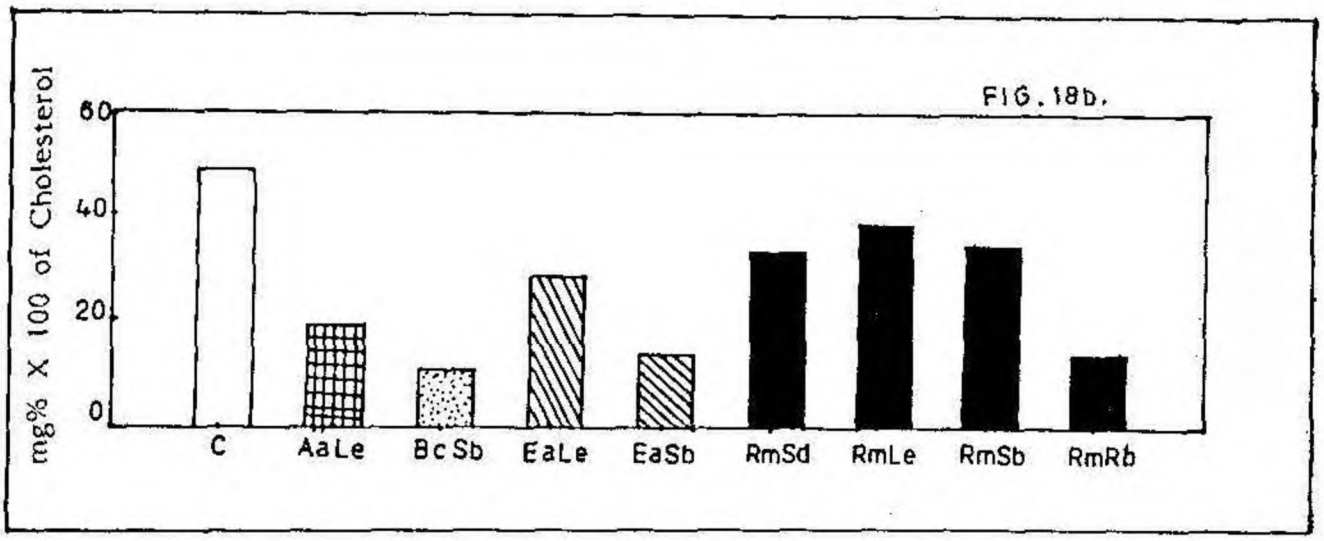

Fig.18b. The cholesterol in muscle tissue of T. mossambica (water soluble)

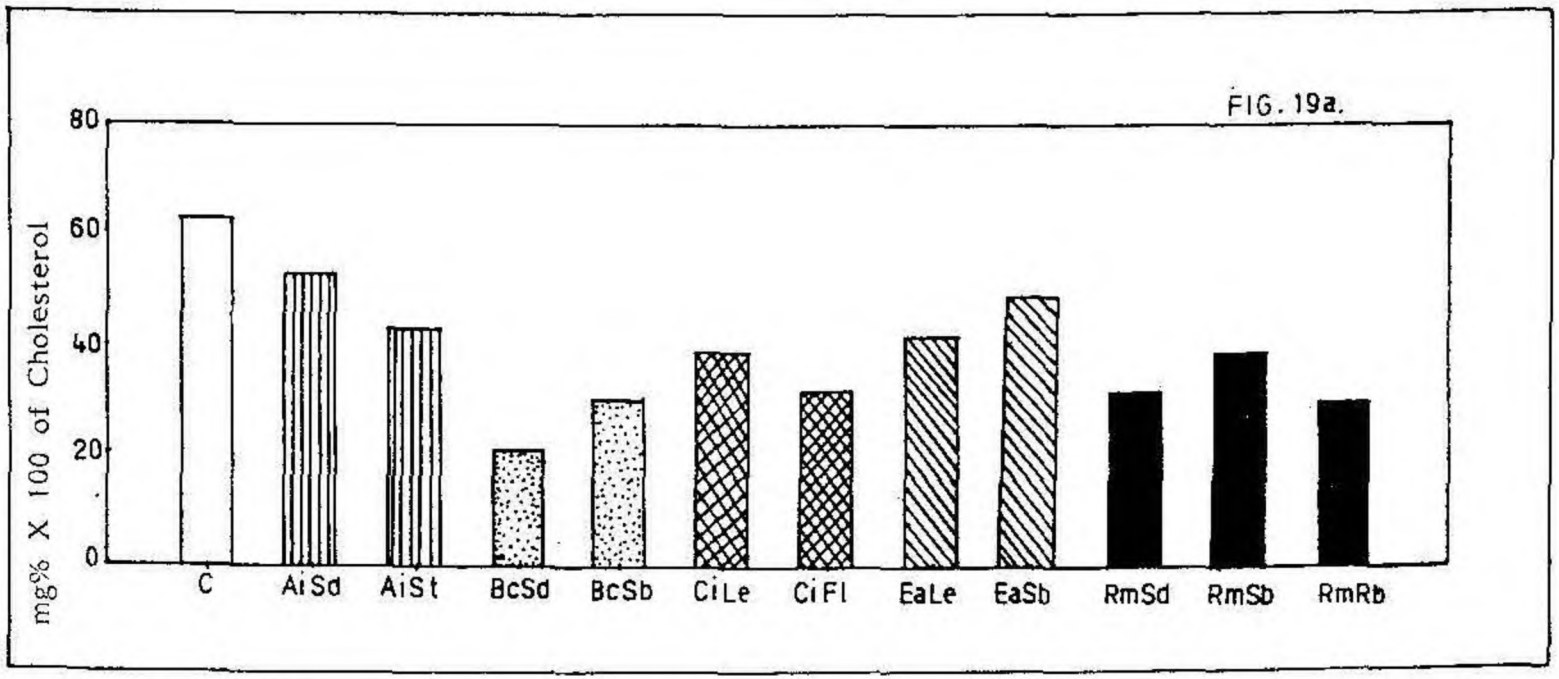

Fig.19a. The cholesterol in muscle tissue of C. chanos (Ethanol soluble)

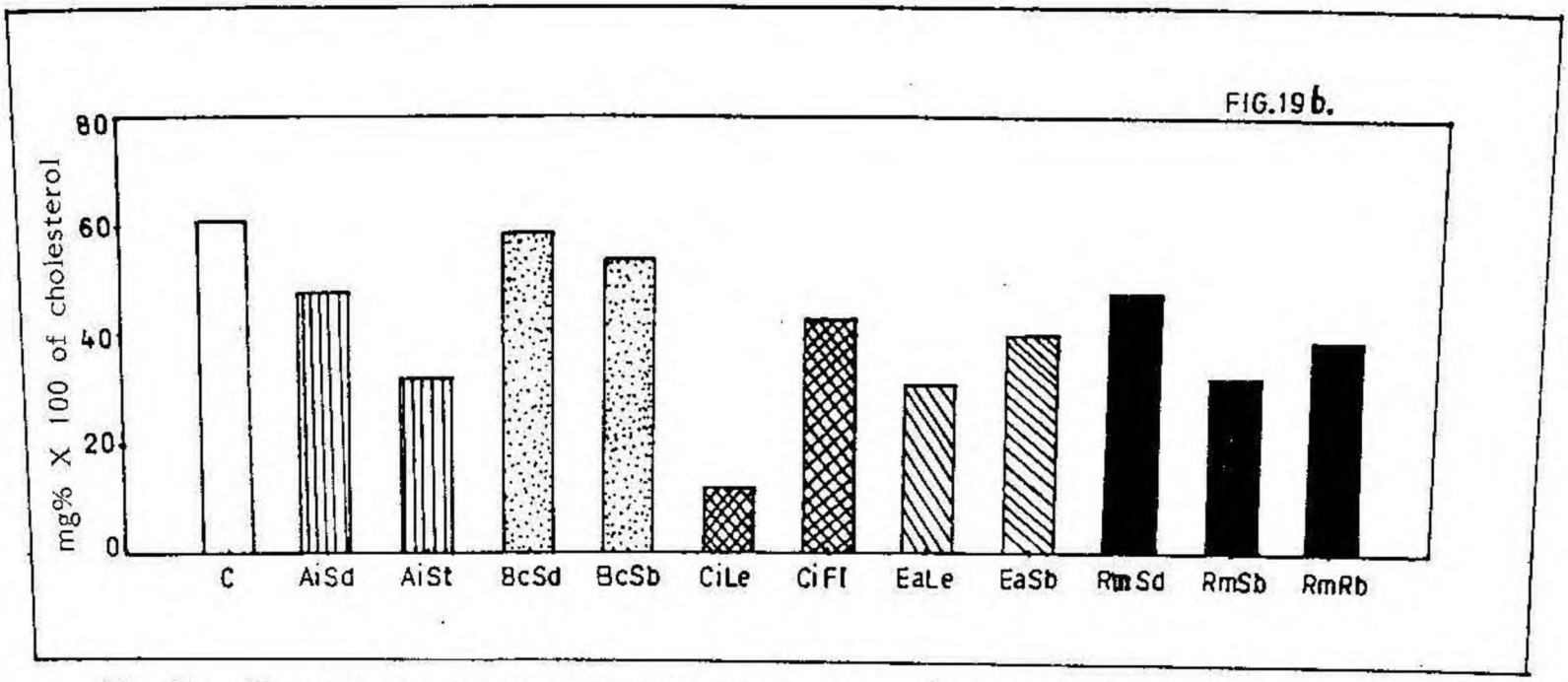

Fig.19b. The cholesterol in muscle tissue of C. chanos (Water solubles)

Explanation of Plates (10a-14b)

Acanthus ilicifolius	1. Seeds 2. Flowers 3. Leaves 4. Stem
Acrostechum aureum	5. Leaves 6. Stem
Avicennia officinalis (Collected from Cochin)	7. Flowers 8. Leaves 9. Stem
A. officinalis (collected from Tuticorin)	10. Leaves
Bruguiesa cylindrica	11. Seeds 12. Leaves 13. Stem bark
Clerodendrum inerme	14. Flowers 15. Leaves 16. Stem
Excoccaria agallocha	17. Leaves 18. Stem bark
Rhizophora mucronata	19. Seeds 20. Leaves 21. Stem bark 22. Root bark.

PLATE 10a & 10b. Spots obtained by paper chromatography of each extract under U.V. light using solvent - n-Butanol: Acetic acid:Water (14:4:50 v/v).

PLATE - 10a

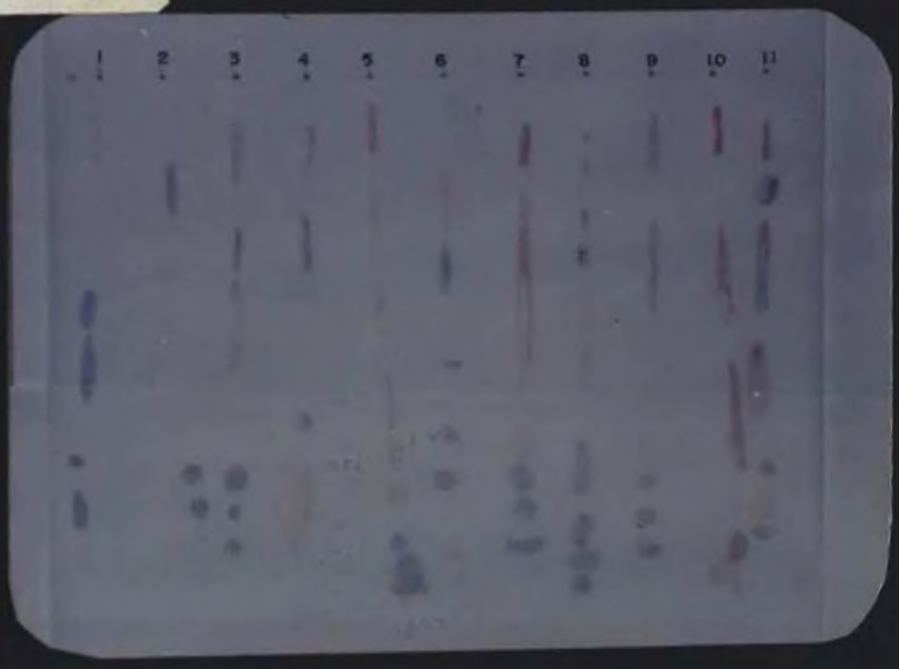

PLATE - 10b

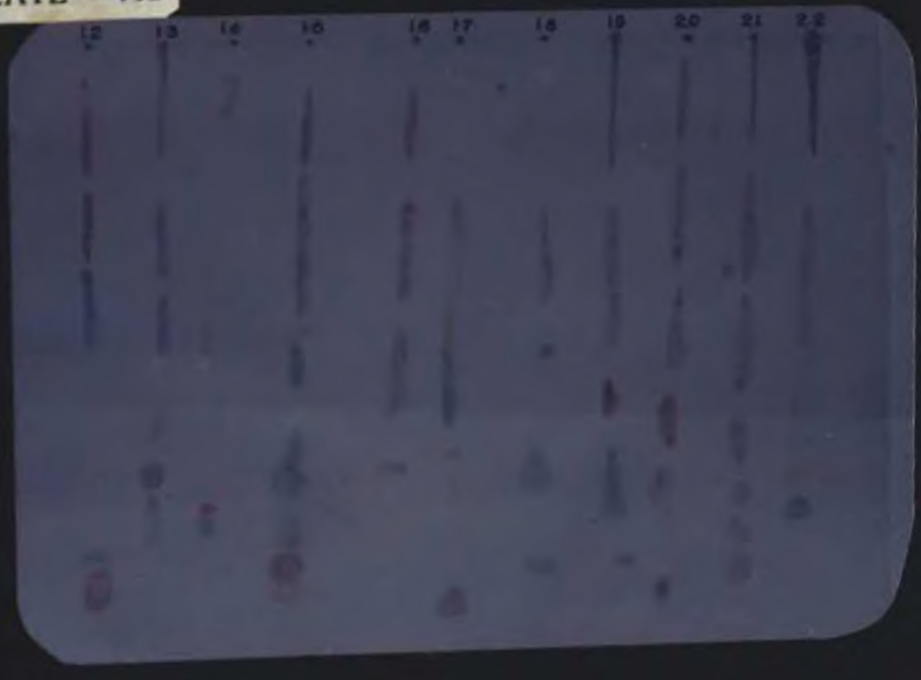

PLATE 11a & 11b & 11c. Spots obtained by paper chromatography of each extract under U.V. light using solvent - n-Butanol:Acetic acid:Water (4:1:1 v/v).

PLATE - 11a

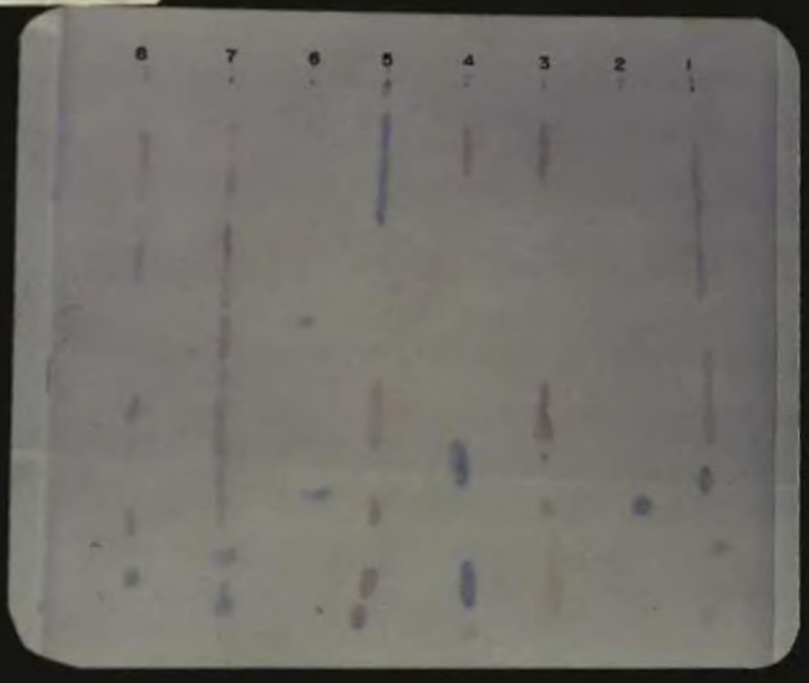

PLATE - 11b

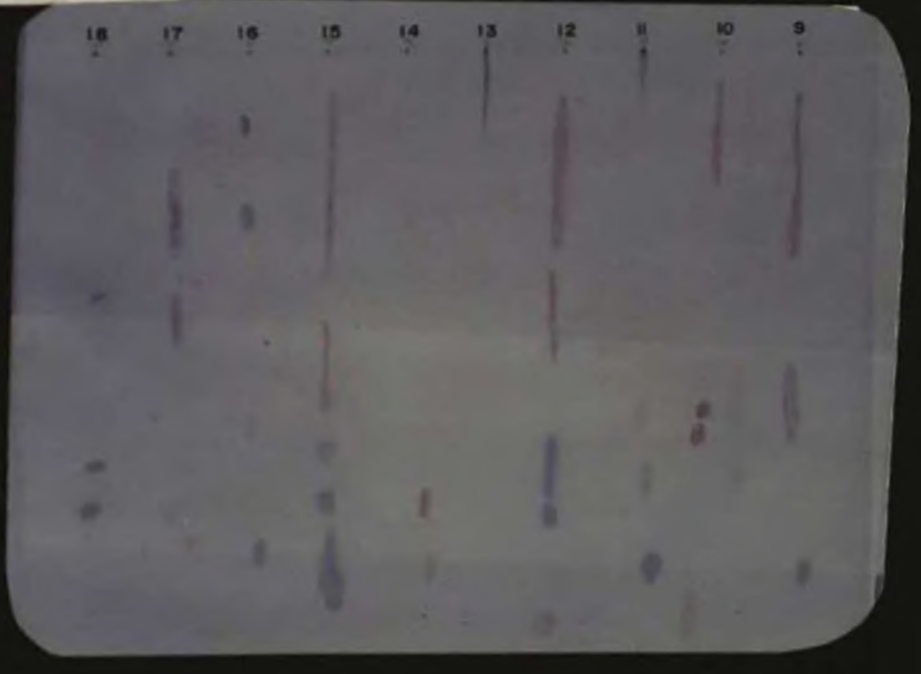

PLATE - 11c

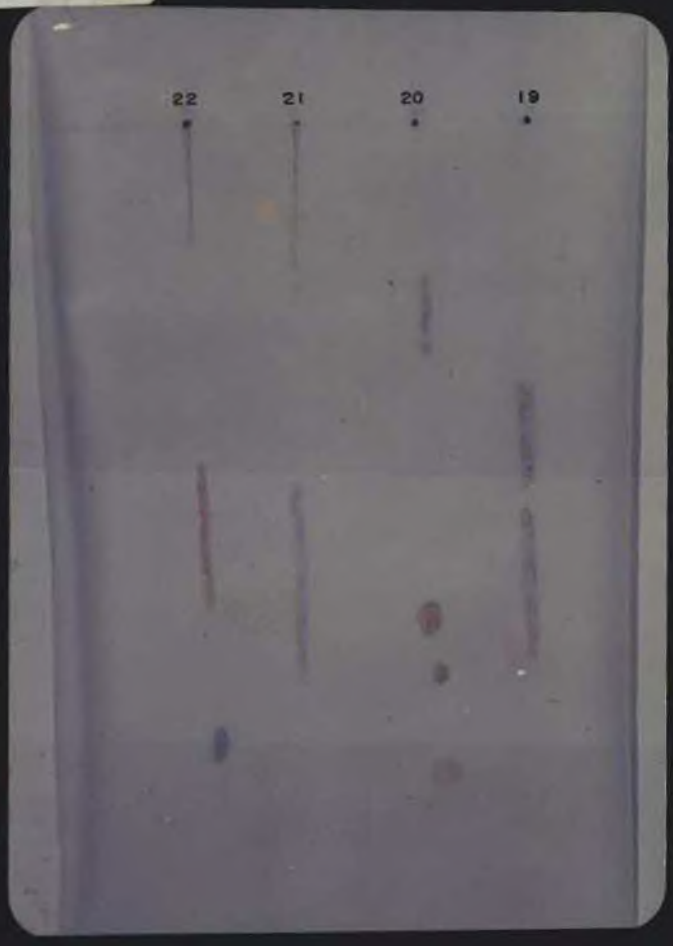

PLATE 12a & 12b. Spots obtained by paper chromatography of each extracts under U.V. light using solvent n-Butanol:Acetic acid :Water (4:1:5 v/v).

PLATE - 12a

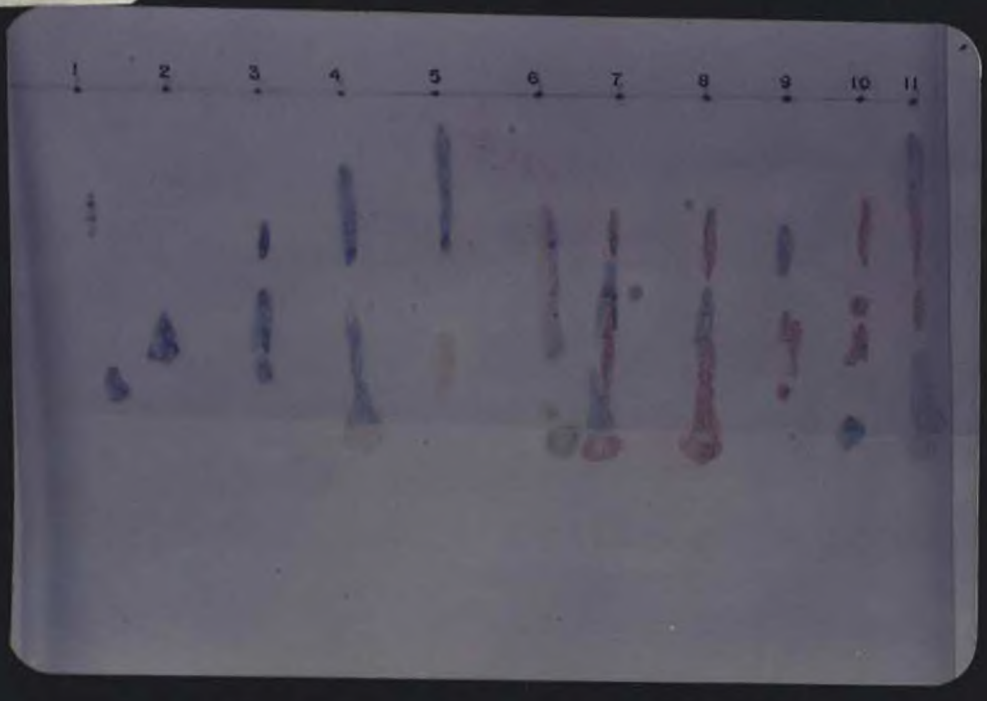

PLATE - 12b

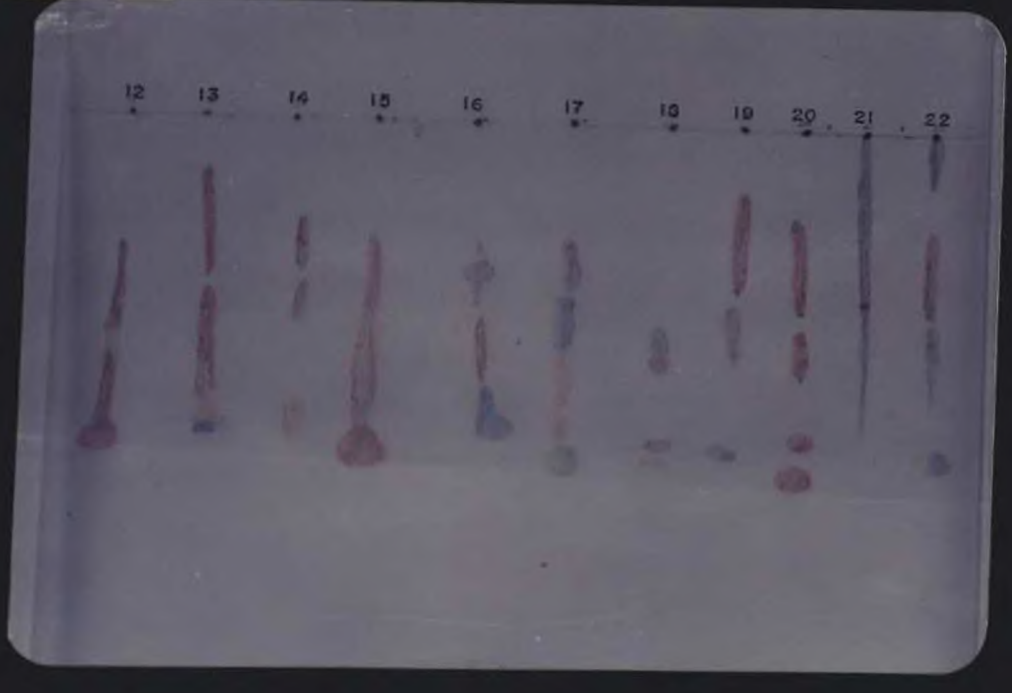

PLATE 13a & 13b. Spots obtained by paper chromatography of each extract under U.V. light using solvent - n-Butanol:Water:ethanol (5:4:1 v/v).

PLATE - 13a

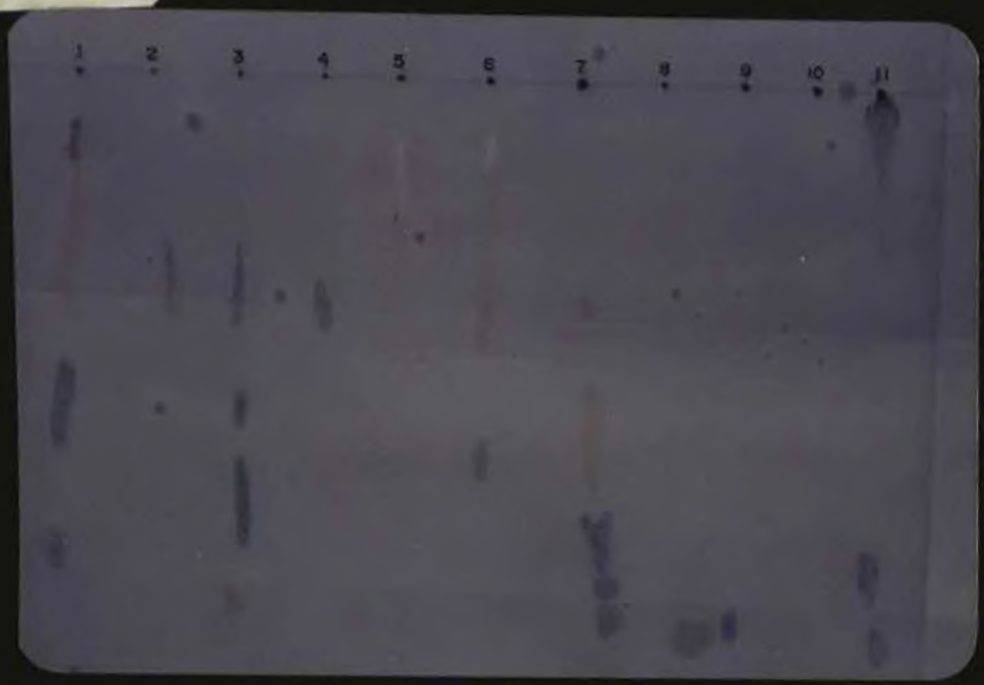

PLATE - 13b

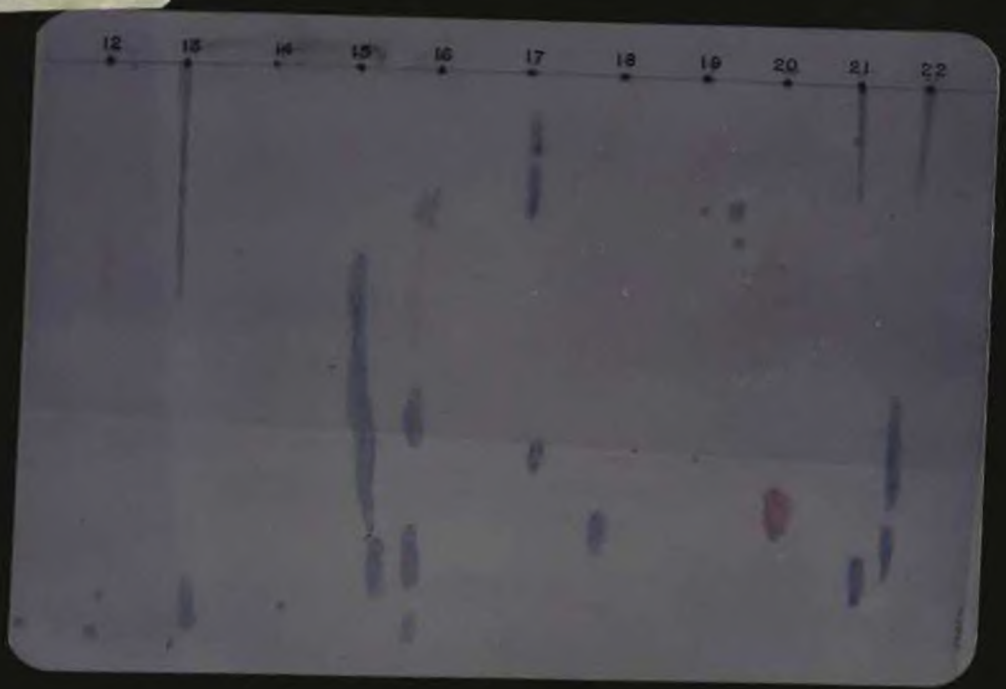

PLATE 14a & 14b. Spots obtained by paper chromatography of each extract under U.V. light using solvent - n-Butanol: Methanol:Water (4:4:1).

PLATE - 14a

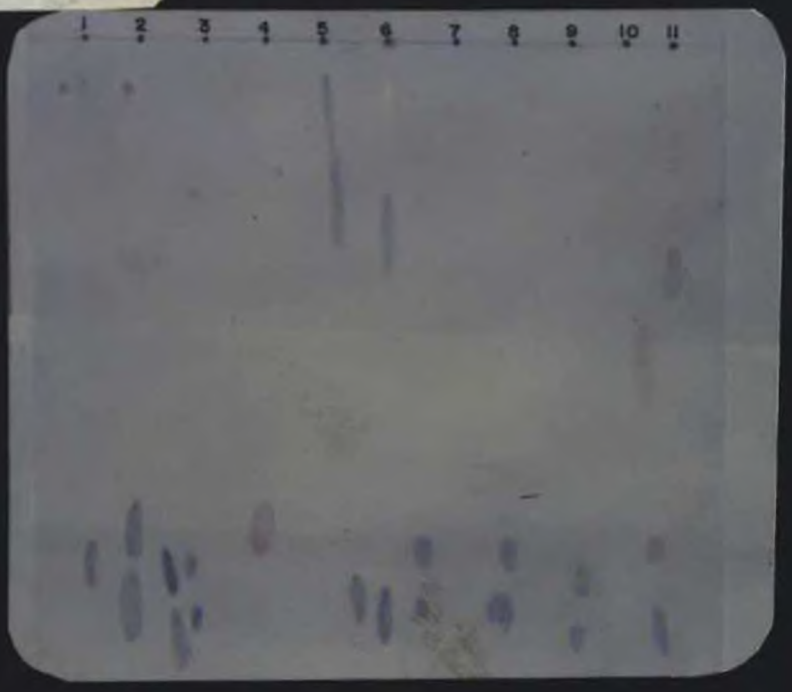

PLATE - 14b

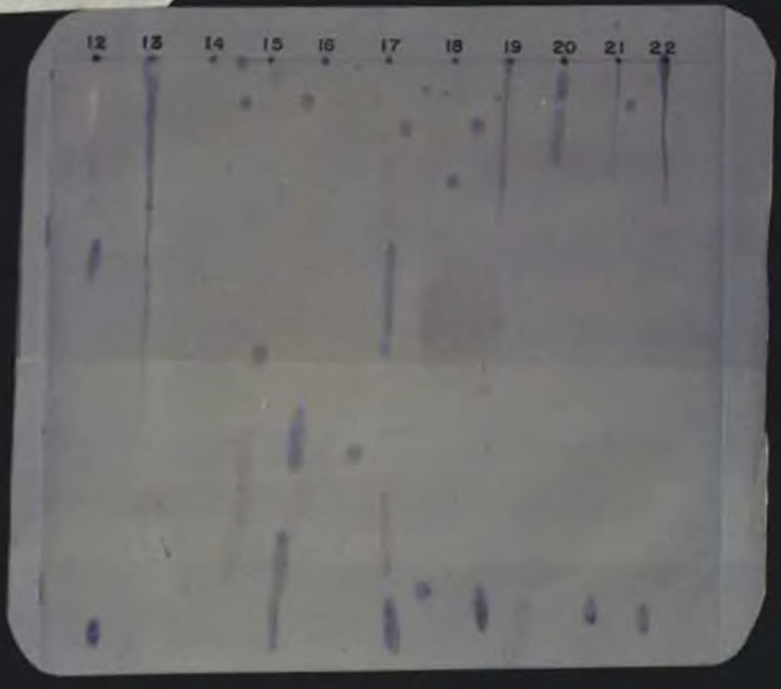

TABLE - 16

PAPER CHROMATOGRAPHY OF ETHANOLIC EXTRACTS

Parts Mangrove species	Rf values in solvent systems									
	n-BuOH:HAC:H_2O 14 : 4 : 50		n-BuOH:HAC:H_2O 4 : 1 : 1		n-BuOH:HAC:H_2O 4 : 1 : 5		n-BuOH:H_2O:EtOH 5 : 4 : 1		B_z : MeOH:H_2O 4 : 4 : 1	
Acanthus ilicifolius										
Seeds	0.14(ly), 0.59(lbl), 0.89(lbl).	0.48(lbl), 0.78(br),	0.24(lp), 0.72(lbl), 0.98(ly).	0.58(lp), 0.85(lbr),	0.38(lp),	0.88(lbl).	0.12(lbr), 0.58(bl),	0.29(ly), 0.82(lbl).	0.75(lbl).	
Flowers	0.22(lbl), 0.83(lbl).	0.76(lbl),	0.69(lbl).		0.68(lbl).		0.35(lp).		0.83(lbl),	0.94(lbl).
Leaves	0.14(lp), 0.42(lp), 0.77(lbl), 0.90(lb).	0.33(lp), 0.5 (y), 0.84(lbl),	0.12(lbr), 0.63(lbr), 0.83(ly).	0.72(lbr), 0.72(lbr),	0.41(lbl), 0.75(lbl).	0.63(lbl),	0.38(lbl), 0.79(lbl),	0.61(lbl), 0.97(ly).	0.81(lbl), 0.84(lbl),	0.91(lbl), 0.89(lbl).
Stem	0.15(lp), 0.66(lp),	0.34(lp), 0.85(ly).	0.12(lbr), 0.89(lbl),	0.70(lbl), 0.99(ly).	0.22(lbl), 0.72(lbl), 0.97(ly).	0.6(ly), 0.87(lbl),	0.40(lbl).		0.74(br).	
Acrostichum aureum										
Leaves	0.10(br), 0.29(y), 0.68(ly), 0.90(lbl), 0.99(br).	0.18(y), 0.63(br), 0.8(ly), 0.94(lbl),	0.15(lbl), 0.71(lbr), 0.89(lbr).	0.57(lp), 0.83(lbr).	0.29(lbl),	0.789(ly).	0.25(ly).		0.01(ly), 0.85(lbl).	0.19(lbl),
Stem	0.26(ly), 0.54(lbl), 0.77(lbl),	0.37(lbl), 0.68(lbl), 0.91(ly).	0.41(lbl),	0.69(lbl).	0.47(ly), 0.87(ly),	0.53(lp), 0.96(lgy).	0.32(ly),	0.69(lbl).	0.01(gy), 0.20(lbl),	0.10(ly), 0.76(lbl).

Contd.....

Avicennia officinalis										
Flowers	0.13(lbr), 0.33(lbr), 0.71(ly), 0.83(lbl),	0.26(lp), 0.51(ly), 0.79(lbl), 0.91(lbl).	0.13(lp), 0.6(lp), 0.92(lbl).	0.32(lp), 0.83(lbl),	0.38(br), 0.69(br), 0.96(lbr).	0.53(lbl), 0.87(lbl),	0.40(ly), 0.81(bl), 0.95(bl).	0.65(ly), 0.91(lbl),	0.78(lbl),	0.87(lbl).
Leaves	0.15(lp), 0.35(lp), 0.56(ly), 0.87(lbl), 0.98(lbl).	0.28(lp), 0.44(ly), 0.76(lbl), 0.93(lbl),	0.23(lp), 0.66(ly), 0.88(lbl).	0.59(lbr), 0.71(lbr),	0.4(lbr), 0.71(br), 0.88(br),	0.59(lbl), 0.76(br), 0.97(br).	0.97(gy).		0.86(lbl),	0.90(lbl).
Stem	0.12(lp), 0.77(lbl), 0.91(lbl).	0.37(lp), 0.84(lbl),	0.22(lbr), 0.90(lbl).	0.60(lp),	0.42(lbl), 0.81(lbr).	0.64(lbr),	0.97(lbl).		0.83(lbl),	0.91(lbl).
A. officinalis (dwarf)										
Leaves	0.12(br), 0.42(br), 0.66(lbr), 0.95(yg).	0.35(br), 0.44(y), 0.9(lbr),	0.13(lbr), 0.64(br), 0.96(ly).	0.60(br),	0.38(br), 0.70(br),	0.59(br), 0.97(gy).	-		0.48(lbr), 0.89(lbl).	0.76(lbr),
Bruguiera cylindrica										
Seeds	0.14(lbr), 0.39(lp), 0.78(br), 0.89(lbl).	0.23(p), 0.62(lp), 0.86(ly),	0.66(ly), 0.94(lbl).	0.78(lp),	0.20(lbl), 0.59(lbr), 0.97(lp).	0.40(lbr), 0.83(lbl),	0.83(lbl),	0.94(lbl).	0.15(ly),	0.35(lbl).
Leaves	0.15(br), 0.48(lbl), 0.97(lbr).	0.32(br), 0.91(lbl),	0.19(lbr), 0.67(lbl), 0.94(ly).	0.43(lbr), 0.75(lbl),	0.51(br), 0.84(br),	0.67(ly), 0.97(lp).	0.40(py).		0.13(ly), 0.91(lbl).	0.31(lbl),
Stem bark	0.36(lp), 0.70(lp), 0.88(lbl).	0.54(lp), 0.80(lbr),	-		0.33(br), 0.87(ly),	0.65(br), 0.92(lbl).	0.92(lbl).		-	

Contd.....

Clerodendrum inerme										
Flowers	0.09(lbr), 0.57(ly), 0.91(lbl).	0.42(ly), 0.87(br),	0.94(ly),	0.86(br) .	0.39(br), 0.94(ly) .	0.57(lbr) ,	-		-	
Leaves	0.16(lbr), 0.89(ly), 0.77(lbl), 0.94(lbr),	0.36(lp), 0.57(lbl), 0.88(bly), 0.98(lbr).	0.22(lp), 0.71(lbl), 0.92(lbl),	0.56(lbr), 0.80(lbl), 0.99(lbr).	0.47(lbr), 0.96(br) .	0.75(lbr),	0.57(bl),	0.88(lbl).	0.70(ly).	
Stem	0.15(lbr), 0.62(lp), 0.87(ly),	0.39(lp), 0.79(lbr), *0.25(lbl).	0.40(lbl), 0.78(ly), 0.92(lbl), 0.30(lbl).	0.70(lbl), 0.30(ly), *0.13(lbl),	0.469(lp), 0.88(lbl),	0.67(lbr), *0.44(lbl).	0.21(lbl), 0.57(bl), 0.93(lbl),	0.35(ly), 0.80(lbl), *23(lbl) .	0.59(lbl), *0.93(lbl).	0.84(by),
Excoccaria agallocha										
Leaves	0.13(ly), 0.45(ly), 0.72(ly), 0.98(lbr).	0.35(lp), 0.61(lbl), 0.79(ly),	0.25(lp), 0.82(ly).	0.44(lp),	0.41(lp), 0.75(ly), 0.96(yg) .	0.16(lbl), 0.87(ly),	0.11(lbl), 0.68(lbl).	0.21(lbl),	0.13(ly), 0.75(lbry),	0.38(lb), 0.89(lbl).
Stem bark	0.09(ly), 0.55(lbl), 0.92(lbl) .	0.37(lp), 0.76(lbl),	0.41(lbl), 0.76(lbl),	0.70(lbl), 0.81(ly).	0.61(lbl), 0.91(lbr) ,	0.68(lbr), 0.96(ly) .	0.80(lbl).		0.84(lbl) .	
Rhizophora mucronata										
Seeds	0.41(lp), 0.79(lbl),	0.63(br), 0.919(lbl).	0.43(lp),	0.64(lp).	0.32(br), 0.91(lbl).	0.59(lp),	-		0.97(lbl) .	
Leaves	0.12(lbr), 0.52(lp), 0.80(lbl), 0.99(lgy).	0.30(lp), 0.68(br), 0.82(ly),	0.28(lp), 0.82(br),	0.73(br), 0.97(ly).	0.39(br), 0.87(lbr),	0.64(br), 0.97(lbr).	0.72(br).		0.04(lbl), 0.91(ly).	0.12(lbl),

Contd.....

Stem bark	0.34(lp), 0.54(lp), 0.71(lp), 0.80(lp), 0.87(lp), 0.92(lp)	0.61(lp)	-	0.87(lb)	0.97(lbl)		
Root bark	0.41(lp), 0.64(ly) 0.76(ly), 0.82(lbl)	0.60(lbr), 0.90(lbl)	0.42(lbr), 0.63(lp) 0.93(lbl)	0.67(lbl), 0.82(lbl)	0.88(lbl)		

n-BuOH-(n-Butanol); HAC-(Acetic acid); H_2O-(Water); EtOH-(Ethanol); MeOH (Methanol)

Colour of spots - lbl (light blue); lbr (light brown); lp (light purple); ly (light yellow); lbr(light brown); br(brown); lv (light violet); lgy (light greenish yellow); gy(greenish yellow); yg(yellowish green); bl(blue) gy(greenish yellow) ; blg (bluish green).

* Additional spots observed on exposure of the chromatogram to ammonia.

DISCUSSION

The observation of these studies have been presented in form of figures, graphs and histograms, so that comparisons and inferences can be drawn easily. The colour of the ethanol extracts is suggestive of their contents. Thus the extract of the seeds and stem bark of B. cylindrica and R. mucronata which were dark or reddish brown is suggestive of the presence of tannins. The greenish or greenish yellow colour of the extract of flowers and leaves is suggestive of the presence of chlorophyll. Similarly the foaming nature of the extract of all parts of B. cylindrica and R. mucronata at the end of distillation, when all the ethanol have been distilled over, is suggestive of the presence of saponins.

Figure 2, depicts the time taken for lethality by the finger lings of L. macrolepis, T. mossambica and C. chanos to the ethanol and water solubles of stem bark of B. cylindrica. It can be noticed that the time taken by ethanol and water solubles for lethality in L. macrolepis and T. mossambica are almost same, where as in the case of C. chanos water solubles are more toxic than the alcohol solubles. This may be due to either more solubility of the toxic compound in water than in alcohol or that there may be an additional toxic compound in the stem bark which is soluble in the aqueous solution and insoluble is ethanol. It is also seen that C. chanos are more

resistant to the toxins of stem bark of B. cylindrica where as L. macrolepis is least resistant.

Figure 3 represents the time taken for lethality of fishes to ethanol and water solubles of the flower of C. inerme. It can be seen from the figure that the time taken for lethality is almost the same by the ethanol and water solubles of flower of C. inerme in the case of L. macrolepis and C. chanos fingerlings. The toxins of flower of C. inerme has no lethal effect on T. mossambica. Of the two species, L. macrolepis and C. chanos the former is affected by toxins more readily than the later.

Figures 4 & 5, represents time taken for lethality by the different fishes to the ethanol and water soluble of leaves and stem bark of E. agallocha respectively. It can be seen from the figure that ethanol solubles of leaves are most toxic to C. chanos less so, for L. macrolepis and least to T. mossambica. L. macrolepis is highly sensitive to the toxins of the ethanol extracts of stem bark of E. agallocha where as T. mossambica and C. chanos are equally less resistant to toxins of the stem bark. It is also seen that the extract of the stem bark of E. agallocha takes less period to produce lethality in L. macrolepis than the extract of leaves. Same is true for L. macrolepis in the case of water extract also. In the case of T. mossambica the ethanol extracts of leaves and stem bark of E. agallocha were more or less equally affective to produce lethality.

In the case of water extract of leaves and stem bark both produced lethality in C. chanos in more or less the same time period where as in the case of T. mossambica the time taken for lethality is more with water extract of stem bark of E. agallocha than that of leaves. In the case of L. macrolepis the case is reverse, that is, the water extract of stem bark of E. agallocha takes less period of time for lethality than that of leaves. Water extract of leaves of E. agallocha produced lethality in T. mossambica and C. chanos in more or less in the same period of time. The water extracts of leaves and stem bark of E. agallocha produced more or less same intensity of lethality in C. chanos.

Figure 6, gives the time taken for lethality to fishes by the ethanol soluble of seeds, leaves, stem and root bark of R. mucronata. It is interesting to note that the fingerlings of C. chanos were not adversely affected by the ethanol extract of leaves of R. mucronata, where as it produced lethality more quickly in T. mossambica than in L. macrolepis. The ethanol extracts of seeds are strongly lethal to L. macrolepis, the time taken for lethality is least compared to all the other extracts of leaves, stem and root bark of R. mucronata. The ethanol extract of seeds produced lethality in T. mossambica and C. chanos more or less within the same period of time. Stem and root bark extracts in ethanol showed almost equal intensity of toxicity in T. mossambica. In L. macrolepis time taken for lethality was slightly higher in the case of the extract of stem bark than that of

root bark where as in the case of C. chanos it was just reverse. Thus in the case of C. chanos the time taken for lethality is slightly less in the case of stem bark than in the case of root bark. In the case of L. macrolepis the order of increasing intensity of toxicity of each extract is seen as leaves, root bark, stem bark and seeds of R. mucronata respectively. In the case of T. mossambica this order is seen to be as seeds, leaves, stem bark and root bark extracts. In the case of C. chanos this order of intensity of toxicity is as stem bark, seeds and root bark even though, all these extracts are more or less equally effective in producing lethality in C. chanos.

Figure 7, represents the time taken for lethality to fishes by the water soluble of each part of R. mucronata. As in the case of ethanol extract of leaves of R. mucronata their water extract is not toxic to C. chanos. The water solubles of leaves show more intensity of toxicity to L. macrolepis compared to T. mossambica. Another interesting feature noted is that C. chanos are more resistant to the water solubles of all parts of R. mucronata and that T. mossambica is least resistant to the water soluble of stem bark and root bark compared to other fishes. In the case of L. macrolepis all the extracts of seeds and root bark of R. mucronata produced lethality within the same period of time where as the extract of stem bark took more time for lethality and the water extract of leaves highest. In general, all the water extracts of the parts of R. mucronata produced lethality in more or less in the

same period of time. In the case of T. mossambica the order of toxicity of water solubles in increasing intensity is as leaves, seeds, stem bark and root bark respectively. In the case of C. chanos this order is as root bark, stem bark and seeds respectively. In general, the C. chanos seemed to be more resistant to the toxins of the water solubles of R. mucronata.

Comparison of ethanol and water solubles of R. mucronata indicate that the later is in general more toxic than in former. Thus, the water solubles of all the parts are more toxic to L. macrolepis than the ethanolic solubles except of seeds and root bark. Ethanol solubles of leaves are more toxic to T. mossambica than the water solubles. The ethanol and aqueous solubles of the root and stem bark are more or less equally toxic to T. mossambica. The aqueous solubles of root and stem bark are less toxic to C. chanos than ethanol extracts. The water and alcohol solubles of seeds, stem and root bark are more or less equally toxic to L. macrolepis extract.

Better understanding of the lethality to fishes is provided by figures 8a to 10b, where the data is represented by histograms. In these histograms the part of the mangroves species showing lethality is presented on X-axis and time taken for lethality is given in Y-axis.

Figures 8a and 8b, represents the toxicity of ethanol and water solubles respectively to L. macrolepis. It is evident from both figures that highest toxicity is shown by the extract of stem bark of

E. agallocha. In the case of ethanol extract least toxicity shown by leaves of R. mucronata and in the case of water solubles by the extract of E. agallocha leaves.

In general, ethanol solubles are more toxic to L. macrolepis than the corresponding water extract except in the case of leaves and root bark of R. mucronata. The decreasing order of intensity of toxicity of ethanol solubles to the fish is as E. agallocha stem bark; R. mucronata seed, B. cylindrica stem bark, R. mucronata stem bark, R. mucronata root bark, C. inerme flower, E. agallocha leaves and R. mucronata leaves. In the case of water solubles this order is as E. agallocha stem bark, B. cylindrica stem bark, R. mucronata seed and root bark, stem bark, leaves, C. inerme flower and E. agallocha leaves.

Figures 9a and 9b, give the toxicity of ethanol and water solubles respectively to T. mossambica. Highest toxicity is given by the water soluble of R. mucronata stem bark. In the case of ethanol soluble R. mucronata root bark shows the highest toxicity. The least toxicity is shown by ethanol and aqueous extract of A. aureum leaves. The water solubles of E. agallocha leaves and R. mucronata seeds show the same intensity of toxicity to T. mossambica. In the decreasing order of intensity of toxicity of ethanol solubles to T. mossambica is as R. mucronata root bark, stem bark, leaves, E. agallocha stem bark, leaves, R. mucronata seeds, B. cylindrica stem bark and A. aureum leaves. This order in the case of water extract is as R. mucronata

stem bark, root bark, seeds, E. agallocha leaves, stem bark, R. mucronata leaves, B. cylindrica stem bark and A. aureum leaves.

Figures 10a and 10b, represents the toxicity of various ethanol and water extracts respectively to C. chanos. Water solubles of E. agallocha stem bark is the most toxic to C. chanos fingerlings. Of all the extracts, the lowest intensity of toxicity is shown by water extract of A. ilicifolius stem to C. chanos. The decreasing order of intensity of toxicity to C. chanos by the ethanol solubles can be arranged as E. agallocha leaves, stem bark, R. mucronata root bark, seeds, stem bark, A. ilicifolius, seeds, B. cylindrica leaves, stem bark, C. inerme leaves, A. ilicifolius stem, C. inerme flower and B. cylindrica seeds. This order in the case of water extracts is as E. agallocha stem bark, leaves, R. mucronata seeds, B. cylindrica stem bark, seeds, A. ilicifolius seeds. B. cylindrica leaves, R. mucronata stem bark, C. inerme flower, leaves, R. mucronata root bark and A. ilicifolius stem. In general water solubles are more toxic to C. chanos than the corresponding ethanol extracts.

Figures 11a and 11b, represents the total free sugar in mg% in muscle tissue of L. macrolepis in cases where lethality was noticed with ethanol and water extract along with the value of control. It can be seen from these figures that the total free sugar content in the muscle tissue are reduced considerably by all the extracts. In the case of ethanol extracts the maximum reduction in the total free sugar content is observed in the fish acted upon by R. mucronata seeds. It is also seen that the free sugar content in the muscle tissues of the L. macrolepis

is reduced considerably by the ethanol extracts of R. mucronata (Seeds, leaves and stem bark). The ethanol extracts of R. mucronata root bark, E. agallocha stem bark, leaves and C. inerme flower reduce total free sugar content more or less to the same extent. In the case of water extracts lowest free sugar content is produced by the extracts of C. inerme flower and E. agallocha stem bark. The extracts of R. mucronata leaves, stem bark, root bark, E. agallocha leaves, B. cylindrica stem bark reduced the free sugar content more or less to the same extent. Comparison of these with figures 8a and 8b reveals that the reduction in total free sugar content does not depend on the time take for lethality by these extracts. It may be probable that the lethality observed may be due to the considerable reduction in total free sugar content and does not depend on the time taken for lethality by these extracts. It may possibly be die to an action similar hypoglycaemia.

Figures 12a and 12b, indicate the total free sugar of the muscle tissues of T. mossambica after lethality to the ethanol and water extracts respectively along with the control. Of all the extracts total free sugar content was reduced considerably in the muscle tissue of T. mossambica by ethanol extracts of R. mucronata leaves. In the case of water extracts also total free sugar was least with the extract of R. mucronata leaves. The reduction in total free sugar in muscle

tissue of fish was more or less uniform by almost all the extracts except by R. mucronata stem bark extract (both ethanolic and aqueous). A comparison of the reaction in L. macrolepis shows that the reduction of free sugar is more intense in the case of L. macrolepis by these extracts than in T. mossambica. A glance of the figures 11a and 11b and 12a and 12b; suggest that the reduction of free sugar content is more in L. macrolepis than T. mossambica. This feature explains the fact, why T. mossambica is more resistant to these extracts than the former. Again, as in the case of L. macrolepis, the time taken for lethality in the case of T. mossambica has no relation whatsoever to their reduction in total free sugar content by these extracts (Figures 9a and 12a, 9b and 12b).

Figures 13a and 13b, show the changes in the total free sugar in C. chanos by all the lethal mangrove extracts (both ethanol and aqueous). A comparison of both figures show that the maximum reduction in free sugar is produced by ethanol extract of E. agallocha leaves where as the ethanol extracts of E agallocha stem bark and water extracts of E. agallocha leaves and stem bark produced more or less same reduction in total free sugar content. The reduction in total free sugar content in C. chanos were more or less same in the case of ethanol and water extracts. It is also seen on comparison with figures for other fishes that the total free sugar content reduction is least in the case of C. chanos, suggesting that C. chanos is more resistant to these extracts and explaining the reason for taking more time for lethality by this fish.

Figures 14a and 14b, give the changes in the total protein content of muscle tissue of L. macrolepis produced by the ethanol and water solubles of mangrove parts, where lethality was observed, along with the control. It is seen from the figures that the variation in protein content is comparable with both extracts and that it is more or less same except that in the case of water extract values are slightly less. In general, water extract seem to produce slight higher decrease in protein contents which indicates that the protein metabolism is affected adversely.

The highest reduction in protein has been observed in the case of ethanol extract of R. mucronata root bark and stem bark in the case of water extract. The least reduction in protein is by the ethanol extract of C. inerme flower and in the case of water extract by that of E. agallocha leaves.

Figures 15a and 15b,show the variation in total protein content of T. mossambica by the ethanol and water extracts respectively along with the control. As in the case in L. macrolepis in all the cases where lethality is observed the protein content is decreased. In general, ethanol and water extract show more or less uniform pictures. The highest protein reduction shown is by ethanol extract of R. mucronata root bark and water extract of E. agallocha leaves. Lowest decrease in protein content by ethanol extract of R. mucronata leaves and water extract of A aureum leaves.

Figures 16a and 16b, show variation in total protein content by the ethanol and water extracts in the muscle tissues of C. chanos. As in the case of L. macrolepis and T. mossambica reduction in protein is noticed in C. chanos also where lethality was observed. In general, the reduction in protein content was more or less same in both extracts. Highest reduction of protein in C. chanos is given by both extracts of R. mucronata seeds and least is shown by both extracts of A. *ilicifolius* stem bark.

Figures 17a and 17b, show the variation in the cholesterol content of L. macrolepis by ethanol and water extract respectively. The cholesterol content of muscle tissue is reduced to maximum by ethanol extract of B. cylindrica stem bark and minimum reduction by ethanol extract of R. mucronata seeds when both the extracts are compared.

Figures 18a and 18b, give the variation in cholesterol content of T. mossambica by ethanol and water extract respectively. In both the extracts the cholesterol content is reduced more effectively than in the case of L. macrolepis. The highest reduction is produced by ethanol extract of E. agallocha leaves and water extract of B. cylindrica stem bark. The least reduction is shown by ethanol extract of R. mucronata stem bark and water extract of R. mucronata leaves.

Figures 19a and 19b give the variation in cholesterol content of C. chanos by the ethanol and water extracts. It is seen that there is only slight variation produced by water extract of B. cylindrica seeds.

The maximum reduction in cholesterol content is produced by water extract of C. inerme leaves. In general, both extracts show more or less same reduction. It is observed that of all the three fishes the cholesterol content is reduced to maximum in T. mossambica.

It is interesting to note that all the extracts which produced lethality to fishes did lower their cholesterol content. Shankarkumar et al.(1989) have noted an increasing lipid content of Pericallia ricini larvae treated with the extracts of the Annona squammosa.

The paper chromatography of the ethanol extracts with various solvent systems indicated that the solvent system namely n-Butanol : Acetic acid : water (14 : 4: 50) is the best giving maximum resolution with maximum number of spots with all the extracts. It is seen from (table 16) that each extract of each part of the plant is a mixture of more than 4 to 7 different chemical components with various Rf values. It is also seen that some extracts of various parts of same plant contain, common spots with same Rf values indicating the presence of same chemical components in them. Even though various solvent systems were used for development of chromatogram with the purpose of identification of various groups of chemical compounds as mentioned in the "materials and methods", the identification could not be carried out due to non-availability of pure authentic specimens of chemical compounds. Further work on these, for separation or isolation of pure of chemical components, was not undertaken simply because of the time limit.

The mangroves contain toxins which are lethal to L. macrolepis, T. mossambica and C. chanos. It is seen that the water and ethanol extract of various parts of mangroves show the same reaction to all the fishes. Thus, there is no difference in the reaction of ethanol and water extracts of same part of mangroves. While some species of mangroves are not lethal to the fishes, they initially produced restlessness, violent behaviour, and escaping tendency and finally they adjusted to the new medium and became normal. The ethanol and water extracts of A. officinalis flower, stem, leaves as well as the extract of dwarf variety of A. officinalis leaves show no adverse action to all the fishes. The species of A. officinalis found at Tuticorin were not growing to the height as in places near Cochin and so it was termed as dwarf variety. The reaction of this variety when compared with that collected in and around Cochin show that there are no regional differences in their action to fishes.

The extracts (both ethanol and water) of A. ilicifolius seed, flower, leaves and stem did not show any adverse action to L. macrolepis and T. mossambica where as the extract of seeds and stem were the only parts lethal to C. chanos.

The ethanol and water extracts of leaves of A. aureum did not have any adverse reaction to L. macrolepis and C. chanos. The ethanol extract and water extract of leaves alone were toxic to T. mossambica. Both water and ethanol extracts of the seeds,

leaves and stem bark of B. cylindrica were lethal to C. chanos where as only the ethanol and water extract of stem bark were lethal to L. macrolepis and T. mossambica. The extracts of seed and leaves did not show any adverse reaction to L. macrolepis and T. mossambica.

The ethanol and water extracts of the flower of C. inerme showed lethality to L. macrolepis and C. chanos while the extracts of its leaves in both solvents showed toxicity to C. chanos. They were not toxic to L. macrolepis and T. mossambica. The extracts of stem bark of C. inerme did not have any toxic action on all the fishes.

The extracts (both ethanol and water) of E. agallocha were lethal to all the fishes. So also the aqueous and ethanol solubles of seeds, stem bark and root bark of R. mucronata were lethal to all the fishes where as the extracts of leaves were lethal to only L. macrolepis and T. mossambica. It is interesting to note that the extract of leaves were not lethal to C. chanos.

Until these studies were made it was generally considered that mangroves provide a nursery ground for fishes like L. macrolepis, T. mossambica and C. chanos. It was also considered that the foliage from fallen leaves of the mangroves in the ecosystem were of nutritive value to the growth of the fishes. The present studies carried out by the author show that only A. officinalis can be considered as of nutritive value for the growth of these fishes. Seeds, leaves, stem and root bark of R. mucronata were lethal to all the fishes except

that leaves were not lethal C. chanos. The leaves and stem bark of E. agallocha were lethal to all the fishes. Further many of the parts of other mangroves were lethal to C. chanos only. In the case of A. aureum the leaves of the plant alone was lethal to T. mossambica only.

Further it is seen that same part of one plant is lethal to some species of fishes while it is non-lethal to other species of fishes. Thus, the general notion that mangroves can be compounded to make artificial nutritive feeds have to be cautiously accepted. The larvae and young ones of a particular species should be tested with the extracts of the part of the mangrove plants for toxicity to these organisms, before considering the same for incorporating into the artificial feeds. Similarly the general notion that mangroves form a nursery ground with nutritive value has to be reconsidered. Much depends on the changes taking place in these water solubles toxin in the aqueous medium of the mangrove ecosystem, the reactions, the biochemical changes involved and their reaction to fishes in these regions.

More detailed studies on the toxic chemical compounds present in these mangrove plants regarding their chemical nature, lethal concentration, their role and fate in the mangrove ecosystem will throw more light and increase the scientific knowledge, regarding the fisheries sustained and nurtured by these regions.

SUMMARY

1. Seeds, flower, leaves, stem and root bark of 'seven species of mangrove namely A. ilicifolius, A. aureum, A. officinalis, B. cylindrica, C. inerme, E. agallocha and R. mucronata were extracted with hot ethanol and water respectively. The parts of the A. offinalis from mangrove area in Tuticorin was also extracted in the same manner to study the regional difference, if any, of this plants.

2. The ethanol and water extract of various parts of different mangroves were studied for their toxic action, if any, to L. macrolepis, T. mossambica and C. chanos.

3. Biochemical studies of the muscle tissue of the fishes which showed lethality to the various extracts were also carried out. These studies carried out were for total free sugar, total protein and cholesterol content.

4. The various ethanol extracts of different parts of mangrove species were also paper chromatographed using five solvent systems and spots determined in ultra violet light as well as after exposure to vapour of ammonia.

5. The studies indicated that, in general, many parts of mangroves tested were mostly toxic and lethal to fishes.

6. Only A. officinalis (all parts) was found to be not lethal to fishes. It was also noted that no regional differences in the reaction of the extracts to fishes.

7. The extract of E. agallocha (leaves and stem bark), seeds, leaves, stem and root bark of R. mucronata were lethal to all fishes. The leaves of R. mucronata extract did not show any lethal action to C. chanos.

8. A. ilicifolius seed and stem bark showed lethality to C. chanos only, where as A. aureum alone showed lethality to T. mossambica only.

9. The seeds, leaves and stem bark of B. cylindrica were lethal to C. chanos while only stem bark was lethal to L. macrolepis and T. mossambica.

10. The flower and leaves of C. inerme were lethal to C. chanos where as flower alone was lethal to L. macrolepis. It is interesting to note that stem bark of C. inerme was not lethal to any of the fishes.

11. The seed and stem bark of A. ilicifolius were found to be lethal to C. chanos only where as flowers and leaves were not showing any lethality to fishes.

12. The ethanol and water extracts did not show any difference in their toxic reaction as far as the lethality is considered to all

the fishes. This is of great importance to the hydrosphere of mangrove ecosystem where the dried and decayed matter of these parts of mangrove may affect the fishery.

13. The biochemical study conducted with the muscle tissue of dead fishes showed that in all cases of lethality by the different extracts there is considerable reduction in total free sugar and that the total protein content in the muscle also showed a decrease. The cholesterol content was reduced only slightly.

14. The paper chromatography of the various extracts showed that most of the extracts were mixtures containing 4 to 7 different chemical components.

15. It is seen that same part of a mangrove plant is lethal to some species of fishes only while it not lethal to other species. Therefore, before considering a mangrove species for compounding artificial feed intended for culture of a particular fish, its toxicity to the larvae and young ones should be studied.

16. More detailed studies on the toxic chemical compounds present in these mangrove plants regarding their chemical nature, lethal concentration, their role and fate in the mangrove ecosystem will throw more light and increase the scientific knowledge, regarding the fisheries sustained and nurtured by these regions.

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